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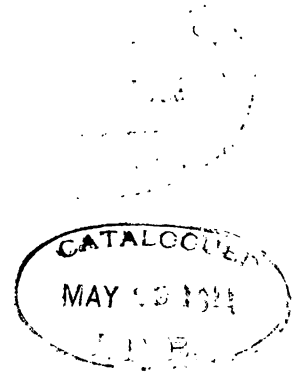

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ON THE USE OF METHYLENE BLUE IN TESTING SEWAGE EFFLUENTS.

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(From the Sanitary Research Laboratory and Sewage Experiment Station of the Massachusetts Institute of Technology.)

THE chief aim of sewage purification processes is to change the organic compounds of sewage into such a stable form that they will no longer tend to undergo offensive putrefactive changes. In special cases, as, for example, when effluents are discharged into tidal waters where shellfish are exposed to pollution, a high percentage removal of bacteria may also be necessary. In most instances, however, the purification plant will have done its work if it produces an effluent free from decomposable organic matter. To what extent this end is accomplished is one of the paramount questions in the control of any plant.

It is more and more clearly recognized that the intelligent operation of sewage works demands systematic analyses of the sewage and effluent at each of the principal stages in the process. The examinations which are made at different plants, however, vary widely in their precision. At the larger works complete analyses are carried out, including total organic nitrogen, suspended and dissolved, free ammonia, oxygen consumed, suspended and dissolved, nitrites, nitrates, dissolved oxygen and total solids, suspended and dissolved, with the loss on ignition. In other cases the effluents are merely examined by a rough test for putrescibility.

There is likely to be an increasing demand for more and more exact scientific control of sewage filters as sanitary standards become increasingly rigorous; rule-of-thumb methods, here as elsewhere, have probably had their best day. No conception can be formed of what goes on in a septic tank or a trickling filter or a contact bed, without knowledge of the amount and nature of the suspended matter. For example, an effluent in itself stable might easily produce in a stream a deposit of solid sludge which would eventually cause a serious nuisance. It is very helpful to the operator of a plant to know the general condition of the nitrogen and the available oxygen content at each stage of the purification process. All the analytical data noted above should therefore be obtained by the analysis of composite samples whenever possible.

The ordinary methods of analysis are, however, insufficient by themselves to furnish all the information necessary with regard to the character of a sewage effluent. Our knowledge of sewage chemistry has not yet made it possible to discriminate clearly between putrescible organic matter and those more stable "humus-like" compounds which do not undergo putrefactive decomposition. With intermittent filters, effluents are obtained which contain only a small amount of organic matter of any sort; and the purity of such effluents is easily demonstrable by oxygen consumed and nitrogen determinations. With effluents from contact and trickling filters, on the other hand, the ordinary analytical data do not offer a complete criterion of quality. An effluent may contain a considerable amount of organic nitrogen and may give a high value for oxygen consumed and yet prove of excellent quality because its organic matter is in a stable form. In such cases it becomes necessary to supplement sanitary analysis by some practical test of keeping quality. Such a test, if its technique is easy, can be made to advantage at more frequent intervals than the more elaborate chemical examinations; and in small plants where the latter are out of the question it alone may prove of considerable value.

Although the terms putrescible and non-putrescible stand for quite definite characteristics, yet the separation of effluents of various degrees of purity into these two classes depends to a considerable extent upon the test employed for the purpose. Crude sewage is

undoubtedly putrescible and a good sand effluent undoubtedly stable; but in that class of effluents in which the chemical analysis yields indefinite information, and in which the putrescibility test is most valuable, the final result is more or less dependent upon the particular technique employed.

Historical.—The most distinctive phenomenon associated with putrefaction is the production of offensive odors. These are due to various substances, generally gaseous, such as ammonia and the higher amido compounds, sulphides, mercaptans, and many others, all of which are associated as a rule with the anaerobic decomposition of proteid matter. It was therefore natural that the earliest tests employed to determine putrescibility should be based upon the development of the odors of these bodies shown in a sample kept in an air-tight vessel for a period of a few days. Such was the test originally devised by Scudder.[1] A glass stoppered bottle was filled completely with the sewage, and stored at a temperature of about 27° C. for several days. If it became dark colored and when opened gave off putrefactive odors it was considered putrescible. This so-called "smell test" was devised and used in 1895. Later, in 1899, at Manchester, the test was placed upon a more scientific footing by making initial and final determinations of the oxygen consumed from permanganate, by the "three-minute cold test;"[2] ferrous salts, hydrogen sulphide, and unsaturated organic bodies which are characteristic of putrefactive changes, are readily and instantly oxidized by permanganate, and hence their presence is readily detected by an increase in the oxygen-consumed figure. Modifications of the test in which the oxygen-consumed figures were determined by longer periods of contact or by boiling with the permanganate, have proved much less satisfactory, since in any such procedure a slight increase is often masked or completely nullified by a simultaneous decrease in the oxidizability of the remaining organic matter.

Later modifications of the test have been made by determining the free dissolved oxygen, and the nitrates and nitrites in the effluent before and after incubation. Such determinations show better than any other the actual course of the changes that are taking place, but the results do not always bear directly upon the question of the stability of the effluent. A marked decrease in these values does, indeed, indicate putrescible or decomposable organic matter; but if, by that decrease, the organic matter be rendered stable before the complete exhaustion of the available oxygen, then it may fairly be said that the effluent as a whole is stable. There are three possible cases. The organic matter of the effluent may be stable or it may be putrescible, i. e., capable of further anaerobic decomposition; in the latter case there may be a sufficient supply of available oxygen, free oxygen, nitrates, and nitrites present to render the organic matter stable, or the amount may be insufficient. Only in this final case is the effluent as a whole putrescible as determined by the older tests. Therefore, in interpreting determinations of oxygen dissolved, nitrates, and nitrites, it is not their diminution but their total exhaustion which indicates putrefaction.

Stoddart[3] made quantitative tests for hydrogen sulphide, and Dunbar and Thumm[4]

[1] *Interim Rep. Royal Commission on Sewage Disposal*, 1901, 2, Question 5988.

[2] FOWLER, *Sewage Works Analysis*, 1902.

[3] *Analyst*, 1901, 26, p. 281.

[4] *Beitrag zum zeitigen Stande der Abwasserreinigungsfrage*, 1902.

tested qualitatively for that gas with lead acetate paper. Both of these modifications, particularly the latter, make the test a much more severe one.

In contrast to such strict standards there has been on the other hand a tendency toward less rigid criteria. Thus Adeney[1] claims that an effluent discharged into a running stream containing itself an abundance of oxygen, should be diluted before testing with the waters of that stream in the proportion of their relative discharges. Clark[2] has used a similar method in a study of the stability of the organic matter in effluents, but as a practical test of the work of a filter it seems altogether too lenient. We have a right to demand that the effluent from a good sewage plant shall not diminish the ratio of oxidizable matter to available oxygen in the stream into which it discharges: by Adeney's test it would pass unless the entire purifying capacity of the stream would be destroyed.

An exhaustive study of the whole subject of putrescibility has recently been made by Johnson, Copeland, and Kimberly.[3] These investigators endeavored to find a relation between the putrescibility or stability of an effluent and its chemical analysis, particularly the oxygen consumed figures on one hand, representing oxidizable material, and the available oxygen in the form of free oxygen, nitrates, and nitrites on the other hand. The data required in establishing such a relation are:

1) The available oxygen in the form of free oxygen, nitrates, and nitrites, and

2) The "consumed oxygen," this being taken as one-fifth of the oxygen-consumed value as determined by the five-minute boiling method.

With these data the following provisional criteria of putrescibility were found to hold for the experiments in question.

When the consumed oxygen value is equal to or in excess of the dissolved oxygen figure and there are no nitrates or nitrites present the sample will putrefy.

When the consumed oxygen value is equal to or slightly less than the amount of oxygen in the effluent in the form of nitrates, nitrites, and dissolved oxygen the sample may or may not putrefy.

When the consumed oxygen value is less than the oxygen contained in the effluent in the form of nitrates and nitrites, under ordinary conditions the sample will not putrefy.

None of the tests for stability so far reviewed have proved wholly satisfactory. The "smell test" is inexact; and at the other extreme the determination of oxygen consumed, dissolved oxygen, nitrates, and nitrites, is too cumbrous a process for routine work. Furthermore, it does not accurately measure the relation of oxidizable matter to available oxygen since the oxygen consumed by permanganate bears a variable relation to the organic matter which is oxidizable under natural conditions. The "Manchester test" is somewhat more satisfactory, but this too yields abnormal results at times and only divides effluents into two rough classes without distinction as to their relative grade.

[1] *Rep. Royal Commission on Sewage Disposal*, 1901, 2, Question 2396.

[2] *An. Rep. Mass. State Board of Health*, 1902, 34, p. 373.

[3] *Jour. Inject. Dis.*, 1906, Supplm. 2, p. 80.

A new putrescibility test, simple in technique, and measuring accurately and delicately the relation between available oxygen and oxidizable matter, has therefore been a desideratum. Such a method appears at last to be at hand in the methylene blue test, first devised by Spitta[1] for the study of stream pollution and later more thoroughly worked up by Spitta and Weldert[2] as a test for sewage effluents. Methylene blue is a commercial dye-stuff of somewhat complex constitution, having the empirical composition $C_{16}H_{18}N_3SCl$. It is prepared technically by the oxidation of dimethyl-p-phenylendiamine with ferric chloride in the presence of hydrogen sulphide. The commercial product is a double chloride of zinc and the above compound. It is an extremely sensitive indicator for hydrogen sulphide and other reducing bodies, being decolorized at once in the presence of even small traces: its decolorization by bacteria has been studied by many observers, the principal of whom are cited by Spitta and Weldert.[3] The technique employed is extremely simple. A small portion of an aqueous solution of the dye (in our experiments 1 c.c. of a 0.1 per cent solution) is added to the effluent in a glass stoppered bottle (250 c.c. capacity in our work), and the sample is then incubated either at 20° C. or at 37° C. The blue color of the solution remains practically unchanged during the period of observation until the available oxygen contained in it is used up and putrefactive conditions arise. At this point the dye is reduced and decolorized. The time required for such decolorization is a quantitative measure of the degree of putrescibility of the sample and the retention of the color for a period of one week or more, at 20° C., or of four days at 37° C., may be taken as an indication of its stability. The criticism of Johnson, Copeland, and Kimberly[4] that "substances other than putrescible organic matters, such as sulphide of iron and hydrogen sulphide," discharge the color of methylene blue before the putrescible matters themselves are able to act, does not seem to us well taken, since such substances are present only when the effluent as a whole is in unstable condition.

Spitta and Weldert used both methylene blue and indonaphtol blue in their experiments, but found the latter difficult to prepare and subject to decomposition. With methylene blue, they tested 46 sew-

[1] *Archiv f. Hyg.*, 1903, 46, p. 113.
[2] *Mitt. a. d. königlichen Prüfungsanstalt f. Wasserversorgung zu Berlin*, 1906, 6, p. 161.
[3] *Loc. cit.* [4] *Loc. cit.*

age effluents of various types, mostly from contact beds and trickling beds, observing the color and odor at intervals, the total period of observation varying from one day to ten. Thirty-two samples failed to decolorize in 12 hours at 37°; and of these only one gave an odor of hydrogen sulphide, and that after two days. The other 14 samples decolorized in periods varying from an hour and a half to nine hours; all of them underwent offensive putrefaction in from 9 to 120 hours. In another series of experiments it was shown that the reduction of the methylene blue always takes place before any odor of hydrogen sulphide became noticeable. In one case the sample was decolorized after three hours and the odor was apparent only after 48 hours. In three other samples decolorization occurred after 18 hours, four days, and four days, respectively, while hydrogen sulphide could not be detected after 10 days.

Both putrefaction and decolorization will naturally vary with the temperature at which the sample is incubated. Spitta and Weldert[1] give the following results, which indicate that putrefaction occurs from two to four times as fast at 37° as at the ordinary room temperature.

TABLE 1.

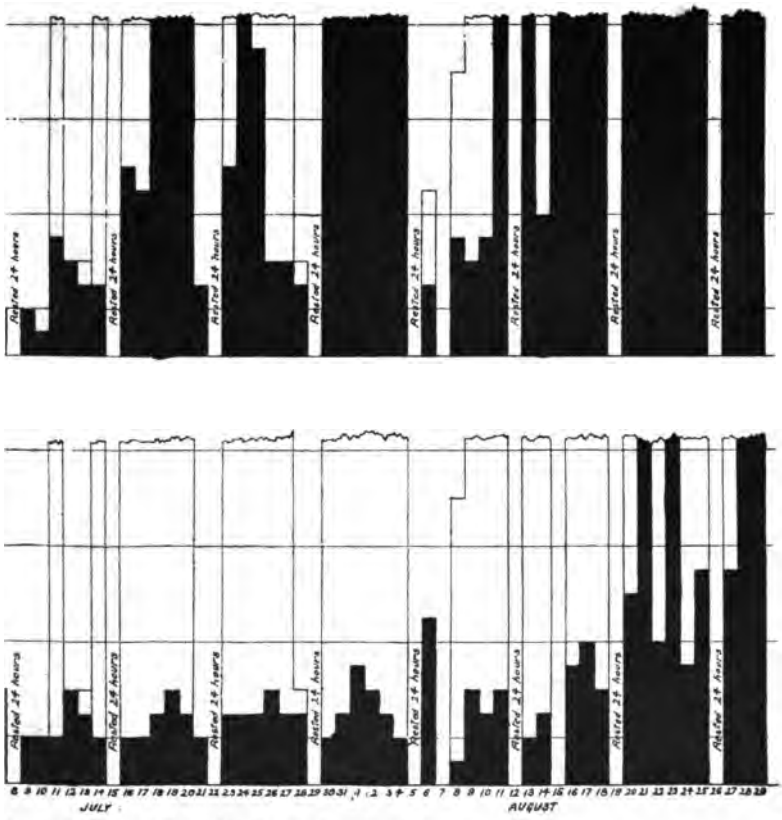
EFFECT OF TEMPERATURE ON DECOLORIZATION OF METHYLENE BLUE.
(Spitta and Weldert.)

Time of Decolorization in Hours.

Sample	22°	28°	37°
1.....	24	6	6
2.....	3	1.5	1.5
3.....	120	96	48

We have compared the decolorization of 64 samples of trickling filter effluent at 20° and 37° in order to obtain further light upon this point. Thirty-four of the samples were not reduced after 14 days at either temperature. In 10 cases the 20° sample retained its color for 14 days while that kept at 37° was decolorized. The period of decolorization at 20° was one day in one case (abnormality due evidently to bad sampling), from three to five days in four cases and from five to seven days in five cases. In the other 20 samples the decolorization times for both temperatures were determined to the nearest half-day and the ratios of the 20° times to the 37° times are tabulated in Table

[1] *Loc. cit.*



husetts Institute of Technology Sewage Experiment Station.

2. The actual number of days varied from half a day to seven days at 20° and from half a day to 13 days at 37° C.

TABLE 2.

RATIO OF TIME REQUIRED FOR DECOLORIZATION OF TRICKLING FILTER EFFLUENTS AT 20° TO THAT REQUIRED AT 37° C.

2.5	2.0	1.6	1.6
1.5	2.6	1.0	0.5
2.7	2.0	4.0	2.2
1.8	2.0	2.0	2.3
1.4	1.9	2.4	2.0

With the exception of two very low ratios (0.5 and 1.0), and one high ratio (4.0), all these figures fall between 1.4 and 2.7 and the average of all is exactly 2.0. It may be considered therefore that methylene blue samples will decolorize at 37° in half the time required at 20°.

It must be remembered in interpreting this table, and all others in which comparative decolorization tests are recorded, that each test must be made in a separate bottle and since the sampling of sewage is never perfect there will be variations due to the chance differences in the several bottles compared. This is particularly true in work like that later cited in Table 5 in which decolorization tests were made in one bottle and analytical data determined on another.

In general it appears from Table 2 that results obtained at 20° bear to those obtained at 37° the ratio of 2 to 1. Stable samples are stable at any temperature. In grading putrescible samples, however, it is evidently necessary to select one temperature and to use it for all comparative work. The temperature of the body has the advantage that it gives results more promptly. Any sample which does not decolorize in four days is certainly stable and a two days' limit would include almost all putrescible effluents. On the other hand, the 20° method, from the very fact that it is slower, permits a more delicate measurement of the relative differences between putrescible effluents of different grades. Decolorization here may occur from the first up to the 14th day. Furthermore, the test can be made, if necessary, at room temperature without an incubator or special apparatus; and this simplicity would be of the greatest importance in small sewage plants without a laboratory or expert supervision. Slight variations from 20° will not introduce a serious

error in the test; and diffuse daylight does not appreciably interfere with it. A series of control experiments, made in daylight, in a box at the room temperature (in summer), and in an incubator at 20°, are recorded in Table 3 and indicate that the test could be made in an ordinary room with fairly controlled temperature. Experiments made during cold weather in a small frame building heated by a stove showed marked abnormalities, on the other hand, and emphasize the fact that larger temperature variations cannot be allowed to go too far.

TABLE 3.

EFFECT OF TEMPERATURE AND LIGHT ON METHYLENE BLUE TEST.

Average Time of Decolorization in Days.

WEEK ENDING	EFFLUENT A			EFFLUENT B			EFFLUENT C		
	Summer Temperature		20°	Summer Temperature		20°	Summer Temperature		20°
	Daylight	Dark	Dark	Daylight	Dark	Dark	Daylight	Dark	Dark
June 27	1	2	2	11	11	7	2	3	3
July 2	3	3	3	12	12	10	3	3	4
July 9	2	2	2	2	3	2	2	3	3
July 16	2	3	2	9	12	10	13	13	13
July 23	2	3	4	7	8	7	14+	14+	14+
July 30	2	5	6	13	14+	13	12	14+	13
August 6	2	3	3	6	6	6	14+	13	13
August 13	3	5	4	13	13	12	13	13	13
August 20	10	5	5	14+	14+	14+	14+	14+	14+

With regard to the significance of the methylene blue test in relation to the specific chemical changes which go on in putrefaction, Spitta and Weldert have little to say beyond pointing out that decolorization takes place only after all the available oxygen has been exhausted. This they illustrate by a single experiment, cited in Table 4.

TABLE 4.

OXYGEN CHANGES IN A TRICKLING FILTER EFFLUENT.

(Spitta and Weldert.)

Days	½	½	1	2	3	4	5	10
Dissolved oxygen; c.c. per L	2.62	1.44	0.50	0.37	....	0.00	....	0.00
Nitrogen as nitrites and nitrates: Mg. per L	10.0	10.0	10.0	....	....	8.0	....	4.0

The methylene blue remained unchanged up to this point.

We have thought it important to study the relation between decolorization and chemical processes somewhat more fully; and the

results of the examination of five samples of trickling effluent are shown in Table 5.

TABLE 5.

OXYGEN CHANGES IN PUTRESCIBLE TRICKLING FILTER EFFLUENTS.

Decolorization of Methylene Blue Indicated by *.

(PARTS PER MILLION.)

SAMPLE	DAYS AT 37°	DISSOLVED OXYGEN	NITROGEN AS		OXYGEN CONSUMED 15 MINUTES COLD
			Nitrates	Nitrites	
1.....	Initial	8.0	3.0	1.0	5.0
	1*	0.0	0.0	0.3	5.0
	2	....	....	0.1	....
2.....	Initial	10.0	3.0	1.0	4.0
	1	1.0	3.0	1.0	4.0
	2	0.0	0.0	1.0	3.4
	3	0.0	...	0.1	4.0
	4	...	...	0.08	4.0
	5	...	...	0.03	3.8
	7	...	...	0.00	4.0
	8	...	...	...	4.8
	9	...	...	...	6.3
3.....	Initial	8.0	3.5	0.5	6.0
	1	1.0	3.0	1.2	4.8
	2	1.2	3.0	1.4	5.7
	3	0.4	3.0	1.0	5.2
	4	0.0	0.0	0.0	5.0
	7*	...	...	...	...
4..... M. B. sample lost	Initial	10.8	4.0	0.3	4.2
	1	1.8	3.0	0.3	4.5
	2	0.2	3.0	0.5	4.4
	3	0.2	3.0	0.1	3.4
	4	0.6	2.5	0.1	2.5
	5	0.0	1.0	0.1	4.3
	6	...	0.0	0.05	3.6
	7	...	0.0	0.0	2.7
5.....	Initial	9.2	3.0	0.6	5.2
	1	1.2	2.5	0.6	5.3
	2*	0.0	0.0	0.0	5.3
	3	0.0	0.0	0.0	5.7
	4	...	...	...	4.6
	5	...	...	...	3.9
	6	...	...	...	6.1
	7	...	...	...	6.9

It is evident that the free dissolved oxygen is absorbed first by the organic compounds present; then the nitrates disappear, and finally the nitrites; and the methylene blue is generally attacked just after the disappearance of the latter. Sometimes, as in sample 3, there is a considerable delay between the disappearance of nitrites and the decolorization of the methylene blue. This may probably occur when the organic matter and the available oxygen are about evenly balanced, so that after the exhaustion of the oxygen putrefactive changes are set up only somewhat slowly. Whenever the methylene blue is decolorized, it may be assumed that all oxygen as nitrates or nitrites has disappeared; and vice versa, when the blue color is retained, traces of these bodies are generally present. This

condition is illustrated in Table 6 for trickling filter effluents which did not decolorize after 14 days at 37°. It will be noticed that these effluents contained a considerable amount of organic matter as measured by oxygen consumed and that in all cases there was a

TABLE 6.

OXYGEN AND OXIDIZABLE MATTER IN STABLE TRICKLING FILTER EFFLUENTS.

(PARTS PER MILLION.)

INITIAL COMPOSITION				AFTER 14 DAYS AT 37°			
Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold	Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold
	Nitrites	Nitrates			Nitrites	Nitrates	
6.8	4.0	5.5	5.6	0.0	0.0	0.0	3.2
2.8	3.0	5.5	8.4	0.3	3.5	1.5	7.6
5.2*	0.0	15.0	4.4	4.0	0.1	15.0	4.4
7.2	5.0	3.0	18.8	0.1	0.4	1.5	8.9
4.4	3.0	3.0	5.2	0.0	0.1	1.5	3.5
7.2	4.0	6.0	16.8	0.1	0.5	5.0	15.8
4.6	3.0	6.0	10.4	0.0	1.5	1.0	12.2
4.4	2.5	5.5	9.6	0.0	0.0	0.0	7.0
4.4	3.0	5.5	8.4	0.2	0.8	1.5	2.0
6.6	1.0	2.0	17.4	0.0	3.0	0.1	2.6
4.2	1.5	3.0	7.2	0.0	1.0	0.0	0.6
5.7	2.5	3.0	8.4	0.0	0.0	0.0	5.2
4.3	3.0	4.0	7.0	0.6	6.0	2.0	7.7

* Sand filter effluent.

marked reduction in oxygen content during incubation. Nitrites and nitrates remained, however, in all but three cases.

This condition may be compared with that of the putrescible effluents in Table 5 and with four other sets of analyses quoted in Table 7.

TABLE 7.

OXYGEN AND OXIDIZABLE ORGANIC MATTER IN PUTRESCIBLE TRICKLING FILTER EFFLUENT.

(PARTS PER MILLION.)

INITIAL COMPOSITION				AGE, HOURS AT 37°	AFTER DECOLORIZATION			
Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold		Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold
	Nitrites	Nitrates				Nitrites	Nitrates	
4.3	2.0	3.0	24.4	24	0	0	1.5	25.2
1.1	2.5	3.0	14.0	12	0	0	0.0	14.0
5.8	1.5	5.0	15.6	40	0	0	0.0	16.0
3.8	1.5	5.0	8.8	120	0	0	0.0	2.8

An interesting practical question is the relation of the methylene blue test to the original constitution of the effluent as determined by chemical analysis. If our methods were sufficiently precise we should

be able to predict from determinations of organic matter and oxygen just what the result of their interaction will be. The condition of an effluent, as indicated by the relation of "oxygen consumed" to available oxygen, corresponds, however, to its putrescibility only roughly. Johnson, Copeland, and Kimberly from their investigations made the generalizations previously quoted as to the relation between the production of odors and the analytical data; but their tables offer numerous exceptions to this general rule. Our experience with the methylene blue test has been much the same. Comparing Tables 5, 6, and 7, a general difference between the stable and putrescible samples is apparent, the former, in Table 6, having on the whole a considerably higher ratio of available oxygen to "oxygen consumed" than the latter as shown in Tables 5 and 7. There are, however, many samples which contradict this general rule, and we have been unable on any basis of calculation to find a marked and constant difference in analysis between stable and putrescible samples. This is still more forcibly brought out in Table 8 which represents the average results of determinations carried on for six months, the analytical data being determined weekly on composite samples, and the methylene blue test made six times a week. The methylene blue samples were kept for 14 days and those which retained their color for that time would probably have done so indefinitely. In calcu-

TABLE 8.

MONTHLY AVERAGE ANALYSES OF TRICKLING FILTER EFFLUENTS AND AVERAGE DECOLORIZATION TESTS.

MONTH	OXYGEN CONSUMED 30 MIN. BOIL	NITROGEN AS		DISSOLVED OXYGEN	METHYLENE BLUE TEST DAYS AT 20°
		Nitrites	Nitrates		
EFFLUENT, FILTER A.					
March	47	0.6	3.6	8.8	7
April	54	0.5	4.6	4.0	7
May	59	1.1	4.5	6.0	3.5
June	40	4.0	3.6	4.4	3
July	43	2.5	3.8	5.0	2.5
August	34	2.5	4.0	4.7	5
EFFLUENT, FILTER B.					
March	28	0.0	4.1	7.5	6
April	41	0.2	5.1	3.8	8
May	46	1.2	4.4	5.1	4
June	31	2.7	3.8	4.1	10
July	36	2.8	5.3	4.8	10
August	33	3.5	4.0	5.8	11

lating averages they receive only the value of 14 days, however. The monthly averages of 10 days or more represent effluents of high stability and those of three and four days, effluents which were distinctly putrescible.

Evidently the methylene blue test does not correspond strictly to our analytical data. There are no differences in the oxygen and "oxygen consumed" values sufficient to account for the wide difference in stability between the effluents for Filter A for June and Filter B for April, for example, or between Filters A and B for August. It is evident that certain factors affect the stability of an effluent which are not revealed by the ordinary methods of sanitary chemistry. These factors must be taken into account in judging of the success of a sewage disposal plant, since the stability of the effluent is precisely the point of greatest importance.

The methylene blue test, during a year's use in the routine study of the trickling filters at the Sewage Experiment Station, has proved itself admirably adapted to the detection of slight variations in this crucial quality of stability. The general results of the test as shown in diagrammatic form on the appended folded sheet, indicate quite clearly its value. Filter A was a trickling filter 8 ft. deep and 10′ × 10′ in area, filled with crushed stone from 1½″ to 2″ in diameter and receiving crude Boston sewage at a rate of two million gallons per acre per day. Filter B was exactly similar but was dosed with the effluent from a septic tank of seven hours' capacity. The block diagrams refer in each case to the effluents as they flowed from the filters; and the outline diagram to the same effluents settled for two hours in tanks of a modified Dortmund pattern. It will be noticed that the two filters gave similar results during the first winter, the unsettled effluents being generally putrescible and the settled effluents generally stable. In April both fell off somewhat, but in May the filter taking septic sewage improved in operation and all through the summer gave effluents which even without sedimentation were generally stable. Filter A, receiving crude sewage, continued to deteriorate. Through June even its settled effluent usually putrefied and in July and August its effluents were stable only after sedimentation. Here were radical differences in the work of two filters, so radical that one might be considered a failure and the other a success. Yet

the analytical data for these same filters, as given in Table 8, offered no adequate hint of the distinction.

We believe, therefore, that some special test for stability is urgently needed in all sewage analysis; and the methylene blue test seems to us to have certain special advantages, and to deserve wider attention than it has so far received. It directly measures the quality of most importance in a sewage effluent, its freedom from the tendency to putrefactive change. It registers this with a greater delicacy than any method with which we are acquainted The range between an effluent which decolorizes in 24 hours and one which retains its color for two weeks makes it possible to distinguish a dozen grades of effluents between the highly putrescible and the certainly stable one. The effect of the Sunday resting-period in decreasing the stability of the effluents shown on the diagram is a striking instance of the way in which this test reveals slight derangements of a delicately organized filter. If results are desired promptly the test may be made at 37° and will then distinguish between a putrescible and a stable effluent in two days. It is so simple in technique as to be peculiarly adapted to sewage works where neither a laboratory nor an expert chemist are available.

THE APPLICATION TO A SOFT-WATER SEWAGE OF DIRECT PROCESSES FOR THE DETERMINATION OF KJELDAHL NITROGEN AND NITROGEN AS FREE AMMONIA

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WHEN the Experiment Station at Waterbury, Connecticut, was started in operation, December, 1905, it was decided to adopt direct processes for the determination of Kjeldahl nitrogen and nitrogen as free ammonia.

After reviewing the literature on the direct determination of Kjeldahl nitrogen, it was decided to apply the method described by Kimberly and Roberts,[1] and used at the Columbus Sewage Testing Station. In carrying out this method under Waterbury conditions, it was soon found that several points of moment under Columbus conditions did not affect the applicability of the method for this soft-water sewage, and hence it was possible to simplify the technique. It is the purpose of the writer to outline briefly the experiments at Waterbury with direct nesslerization in Kjeldahl nitrogen work, together with a brief discussion of the applicability of the standard method for the direct determination of nitrogen as free ammonia, in each instance showing by comparative data the relation between results by direct and distilled methods.

THE DIRECT KJELDAHL PROCESS.

In developing the direct Kjeldahl procedure for the soft-water sewage of Waterbury, attention was directed to the question of the interference of calcium and magnesium, which Columbus experience indicated to be the controlling feature in successful direct nesslerization. Analysis showed, however, that neither calcium nor magnesium was present in the Waterbury sewage to such an extent as to cause turbidity in a nesslerized tube.

After eliminating from the discussion the calcium and magnesium factors, the next step was to determine the effect of different grades

[1] *Jour. Infect. Dis.*, Supplm. 2, 1906, p. 109.

of caustic soda upon the color produced by Nessler's reagent; for, as the Columbus results indicated, the purity of the caustic soda used in neutralizing the acid digestates controls in a large measure the clearness of ammonia tubes when nesslerized.

Caustic soda from sodium was tried first and with highly satisfactory results, but the cost seemed too high for routine laboratory work. Caustic soda by alcohol was next used. No cloudiness resulted, but, with increasing quantities of the alkali, the depth of the color produced increased slightly, as has been found by former experience.[1, 2]

The grade of caustic soda commonly known as "Purified Sticks" did not give satisfactory results. When fully purified the "blank" was high and the amount of preparatory treatment necessary was not commensurate with the difference in cost between this product and that by alcohol.

THE WATERBURY METHOD.

Having satisfactorily disposed of the question of turbidity and that of the most suitable grade of caustic soda, our attention was directed so to modify details of the method used at Columbus as to develop a process which would be practical and reliable under Waterbury conditions. After some experimentation the method finally assumed the following definite form.

Method.—A suitable portion of the sample (50 to 100 c.c.) is placed in a 300 c.c. Kjeldahl flask, diluted to a volume of 150 c.c. and boiled down to 25 c.c. to insure the removal of the nitrogen as free ammonia. The usual amount of sulphuric acid is then added, and the digestion is then carried to completion. After cooling, the contents of the flask are removed into a 100 c.c. graduated flask, thoroughly shaken, and from the sample there are removed 50 c.c. and placed into a second 100 c.c. flask. The caustic soda is now added, just short of the neutral point; the flask is cooled and the digestate made alkaline, adding in excess not more than 1 c.c. of a 25 per cent caustic-soda solution. The solution is then poured into a dry four-ounce bottle which is tightly stoppered, and set aside for twenty-four hours. During this period it is advisable to agitate the bottle slightly, so as to detach any suspended particles that may cling to the sides.

At the end of 24 hours, or at least of 12 hours, 10 c.c. of the clear supernatant liquor are then removed and placed into a 50 c.c. Nessler tube to which there has been previously added about 25 c.c. of ammonia-free water.

The tubes are next filled to the mark and are mixed by inverting three or four times; the tubes are then nesslerized and read in the usual manner.

[1] NESSLER, *Tiemann-Gartner's Handbuch*, 1905, p. 114.

[2] HAZEN AND CLARK, *Amer. Chem. Jour.*, 1890, 12, p. 425.

To compute the results, which, of course, are in terms of organic nitrogen, it is convenient to use the formula worked out by Kimberly and Roberts:[1]

$$\frac{(N \times 20) - \text{Blank} \times 1{,}000{,}000 \times .00001}{S};$$

where N = c.c. standard reading, S = c.c. sample digested.

DISCUSSION OF DETAILS OF METHOD.

In the Columbus method no provision was made for first removing the free ammonia before the digestion. In applying the Columbus method to the work at Waterbury, it was not found practicable to obtain results comparable with those of the distillation process, nor with each other, for the probable reason that the quantity of sample taken for analysis, especially in the case of strong sewages, was so small as to introduce a large factor of error. To avoid this, it was decided to remove by boiling the nitrogen as free ammonia, thus permitting the use of a greater quantity of the sample. In the opinion of the writer, the necessity for the removal of the nitrogen as free ammonia before digestion, is probably controlled in a large measure by the relative amounts of nitrogen in that form and as organic nitrogen, although he favors the complete removal of the nitrogen as free ammonia.

It was found important to measure accurately the quantity of caustic soda to be used in neutralizing the acid digestates. For this purpose it was found convenient to arrange a four-liter bottle of the caustic soda in such a position that the solution could be blown into a burette whereby the amount of alkali could be accurately measured.

The necessity for using in excess not more than 1 c.c. of a 25 per cent caustic-soda solution lies in the fact that tubes which contain a large excess of alkali do not mix well, on account of the difference between the specific gravity of the dilution water and that of the alkali; and further because samples which are barely alkaline never become entirely free from suspended particles. According to the writer, a failure to observe either one of the precautions is sure to result in cloudy tubes.

COMPARISON OF RESULTS BY DIRECT AND DISTILLED METHODS.

To judge of the feasibility of adopting the direct process, as a routine procedure, a large number of analyses were made, comparing

[1] *Jour Infect. Dis.*, Supplm. 2, 1906, p. 117.

the results of the direct process with those of the distilled, as obtained according to the standard procedure. Representative analyses are listed below, and a study of the results indicates that the direct method is capable of affording very satisfactory results, which agree closely with the results of the distillation method.

TABLE 1.

EFFICIENCY OF DIRECT PROCESS FOR DETERMINING KJELDAHL NITROGEN AS COMPARED WITH THE DISTILLATION METHOD.

SOURCE OF SAMPLE	ORGANIC	
	Direct	Distilled
Crude sewage	7.0	7.0
	7.3	7.4
	7.0	6.9
Septic sewage	18.0	19.0
	16.4	16.3
	11.5	12.2
Sprinkling filter effluent	15.0	14.8
	10.7	11.2
	8.7	8.4
Effluent contact filter	12.0	12.0
	10.5	11.0
	9.8	9.5
Sand filter effluent	7.8	8.0
	5.2	5.0
	9.0	9.4

APPLICATION OF THE DIRECT KJELDAHL PROCESS TO THE DETERMINATION OF NITROGEN IN SEWAGE SLUDGE.

Following the general scheme outlined for the analysis of samples of sewage and effluent, the direct Kjeldahl process was applied to sludge analysis. The procedure adopted was as follows:

An amount of sample is taken that will yield from 0.005 to 0.02 gm. of nitrogen; usually about 0.5 gm. of dried sludge lies within the above limits. Twenty c.c. of sulphuric acid are then added and the digestion continued until the digestate becomes clear. After cooling, the contents of the flask are rinsed into a 250 c.c. graduated flask, and made up to the mark with ammonia-free water. Twenty-five c.c. of this solution are next introduced into a 200 c.c. flask made slightly alkaline with caustic soda, exercising precautions similar to those necessary in sewage work, the solution made up to 200 c.c. poured into an eight-ounce bottle and allowed to stand for 24 hours. Five c.c. of the clear supernatant liquid are then removed and nesslerized in the usual way.

Very satisfactory results have been obtained by this method, as indicated by the table below in which there are presented the results of duplicate analysis of sewage sludge.

TABLE 2.

REPRESENTATIVE DUPLICATE RESULTS OF NITROGEN DETERMINATION IN SEWAGE SLUDGE.

SOURCE OF ANALYSES	DUPLICATE ANALYSES	
	Per Cent Nitrogen	Per Cent Nitrogen
Grit chamber sludge	4.74	4.50
	1.66	1.24
	1.15	1.34
Settling tank sludge	2.37	2.40
	2.04	1.85
	1.98	2.10
Septic sludge	1.60	1.60
	1.60	1.60
	2.00	1.97

NOTES ON THE DIRECT DETERMINATION OF FREE AMMONIA BY THE STANDARD METHOD.

In determining nitrogen as free ammonia by a direct process, the method outlined by the Committee on Standard Methods of the Public Health Association has been followed, except that the sewage is filtered before the addition of the caustic soda and the copper sulphate.[1, 2]

Necessity for filtration.—The idea of a preliminary filtration of the sample was suggested by the fact that when the unfiltered sewage was treated there remained in suspension from 30 to 50 parts of colloidal matter, as against from 10 to 15 parts, in case a preliminary filtration was included in the technique.

From experience gained during the work at Waterbury it has been found that in case the supernatant liquid, from a direct nitrogen as free ammonia determination, contains more than 30 parts per million of colloidal matter; high results will be obtained even though but 5 c.c. of the supernatant are removed and then diluted 10 times. As above mentioned, this feature is entirely eliminated by passing the sample through filter paper before beginning the determination.

Entrainment.—In all direct processes there is of course afforded an opportunity for slightly low results, owing to the entrainment of ammonium salts by the forming of precipitates. In agreement with the results obtained by other investigators,[3] the Waterbury results

[1] FARENSTEINER, BUTTENBURG, AND KORN, *Leitfaden f. d. chem. Untersuch. von Abwässer*, Hamburg, 1902

[2] PHELPS, "Determination of Ammonia in Sewage," *Jour. Infect. Dis.*, 1904, 1, p. 327.

[3] KIMBERLY AND ROBERTS, *Jour. Infect. Dis.*, Supplm. 2, 1906, p. 119.

indicate that the nitrogen lost by this means may be considered a negative quantity. This, in the opinion of the writer, may be inferred from the table comparing the results of Kjeldahl nitrogen determinations by direct and distilled processes.

As evidence to support the view that the entrainment factor is by no means of practicable moment, there is presented below a table showing comparative results of nitrogen as free ammonia determined by the direct and distillation processes respectively.

TABLE 3.

RESULTS OF NITROGEN AS FREE AMMONIA BY DIRECT AND BY THE DISTILLATION PROCESSES

Source of Sample	Direct	Distilled
Crude sewage	17.5	18.0
	10.3	10.3
	17.0	16.8
Septic sewage	12.0	12.0
	9.5	9.4
	9.8	8.9
Sprinkling filter effluent	6.2	5.8
	6.1	6.1
	5.9	5.6
Sand filter effluent	2.5	2.0
	3.8	4.5
	2.4	2.4

CONCLUSIONS.

In conclusion it may be said that the direct process for the determination of Kjeldahl nitrogen, and that for the determination of nitrogen as free ammonia, have been entirely satisfactory under Waterbury conditions. Out of some 4,000 or 5,000 analyses, not more than 10 per cent of the determinations have been in error, due to cloudy nesslerized tubes, and in almost every case the cause of turbidity could be traced to some error of technique and not to any inherent fault in the process itself. Furthermore, by adopting the above methods it has been possible to increase the number of samples analyzed, and to obtain just as efficient results as if the distillation method had been used.

Acknowledgments are here given to Mr. William Gavin Taylor, Resident Engineer, for the many courtesies extended the writer during the preparation of this paper and especially to Mr. R. A. Cairns, City Engineer, Waterbury, Connecticut, for permitting the printing of these studies before the appearance of the Experiment Station Report.

EXPERIMENTS WITH METHODS FOR THE RAPID DETECTION OF GELATIN LIQUEFACTION IN THE DETERMINATION OF *B. COLI*.

STEPHEN DEM. GAGE.

(From Lawrence Experiment Station, Lawrence, Mass.)

IN determining the presence or absence of bacteria of the colon type by the methods approved by the Committee on Standard Methods of Water Analysis, the procedure consists essentially of three steps: first, a detection of gas-producing organisms by means of a fermentation test; second, the separation of acid-fermenting types from other types by plating on litmus-lactose agar and the transfer of typical colonies to agar streaks; third, the identification of the cultures so isolated by their cultural and biochemical reactions when grown in dextrose broth, milk, nitrated peptone solution, Dunham's solution, and gelatin. Since cultures of the colon type grow rapidly and luxuriantly at 40° C., it is possible to complete the first two steps and the identification tests for fermentation, milk coagulation, and nitrate reduction within four days, and the indol test within seven days from the time that the examination of the sample was begun. Since the ordinary culture gelatin does not remain solid at temperatures as high as 40° C., and as the liquefaction of gelatin proceeds very slowly in the case of many common types of bacteria, which can best be excluded from the colon type by this test, it is customary to incubate the gelatin cultures at 20° C. for a period of 14 days. The delay of 10 days required to obtain the liquefaction tests after the other tests are complete is objectionable in many cases where it is desirable that analyses should be reported at the earliest possible date, and many methods have been proposed by which this delay might be obviated. A number of these methods, all based upon the assumption that because the types of bacteria commonly encountered in testing for *B. coli* grow more rapidly and more luxuriantly at body temperature than at room temperature the production of proteolytic enzymes will be correspondingly rapid at the higher temperature, have been investigated at the Lawrence Experiment Station during the past eight

years. Since data which tend to disprove this fundamental assumption must apply with equal force to all the methods tested, statistics regarding only one of the methods will be presented, together with a general statement of the results obtained in tests of the other methods. It is not the purpose of the present paper to enter into a general consideration of the properties of bacterial enzymes, or the conditions under which those properties are manifest, except in so far as they may apply to the specific problem of obtaining a rapid diagnosis of gelatin liquefaction in routine *B. coli* tests.

Substitutes for gelatin.—The three media commonly used for liquefaction tests in bacteriology are gelatin, blood serum, and casein. At the suggestion of the late Dr. Wyatt Johnston, the writer studied the possibility of substituting liquefaction tests on Löffler's blood serum at body temperature for gelatin tests at room temperatures some years ago. In that investigation it was found that a considerable percentage of cultures liquefying gelatin in 14 days at 20° C. failed to liquefy serum at 40° C. in four days or less, although a luxuriant growth usually occurred in 18 to 24 hours. In an investigation of the species of bacteria commonly found associated with *B. coli* at Lawrence, out of 46 different species isolated and studied, six only were found which liquefied both gelatin and serum, while 12 liquefied gelatin but did not liquefy serum, and five liquefied serum but did not liquefy gelatin. In other words, we would have obtained a correct report with only 13 per cent of the species, from liquefaction tests on serum, when the tests were made at 20° C., and it is quite probable that a shorter incubation at 40° C. would have shown even a smaller percentage accuracy.

The great difficulty in preparing a casein medium upon which liquefaction tests can be accurately made is a serious objection to its use. No direct investigation of casein as a substitute for gelatin has been made at Lawrence. In the study of species of bacteria mentioned above, however, only five species liquefied both casein and gelatin, and 13 species which liquefied gelatin failed to liquefy casein.

The investigations of Malfitano,[1] published in 1903, definitely established the fact that the liquefaction of albumen and of gelatin

[1] *Compt. rend. de la Soc. de Biol.*, 1903, 55, p. 843.

was due to entirely separate and distinct enzymes, and show the futility of attempting to substitute serum or casein for gelatin.

Modified gelatin.—A number of processes for preparing a gelatin medium which should remain solid at 40° C. and at the same time embody the properties of the ordinary culture gelatin have been tried.

Mixtures of gelatin and agar were first proposed by Hiss[1] for separating the typhoid, colon, and paracolon types. Attempts to use media of this type for liquefaction tests at 40° C. proved unsuccessful for the reason that if the proportion of agar present was great enough to make the media even reasonably stiff, liquefaction occurred very slowly or not at all, while a fluid or semifluid mixture had no advantages over gelatin alone.

Mixtures of gelatin and serum were suggested by the gelatin-agar mixtures. A number of such mixtures were tried with little success, cultures which liquefied gelatin at 20° C. frequently failing to show any indication of liquefaction when grown on these media for four days at 40° C. Any liquefaction results obtained on such media would, of course, be subject to the same inaccuracy as in the use of serum alone.

In 1901 a German investigator[2] suggested that the melting-point of gelatin might be raised to above 40° C. by the addition of small quantities of formalin without impairing its value as a medium for the cultivation of bacteria. Many attempts have been made at Lawrence to prepare a satisfactory medium in this manner. The problem is to add just the proper amount of formalin. If too much be added the gelatin becomes very hard and liquefaction proceeds very slowly, any considerable excess of formalin will effectually prevent the development of bacterial growth, while if too little be added the melting-point will not be raised sufficiently to prevent the gelatin becoming fluid at 40° C. The process might possibly be worked on a commercial scale to produce a bacteriological gelatin, but its application in the laboratory appears to be impractical.

Attempts to prepare gelatin media with a melting-point between 40° and 50° C. by the addition of alcohol or tannic acid were also attended with little success, the difficulties encountered being similar to those in the formalin process.

[1] *Jour. Med. Res.*, 1902, 8, p. 148.

[2] *Centralbl. f. Bakt.*, 1901, 30, Abt. 1, p. 368.

Preliminary cultivation methods.—The small success obtained in the experiments previously described led to a radical change in procedure. Cultures of liquefying bacteria were seeded into broth and incubated 24 to 48 hours at 40° C., 1 c.c. of the luxuriant culture so obtained was mixed with 5 c.c. of fluid gelatin, and, after solidifying the gelatin, the culture was incubated at 20° C. By such procedure it was hoped that the introduction of a large number of bacteria, together with whatever enzymes might have been formed in the broth, would result in liquefaction becoming manifest in a shorter period than would be the case when stab cultures were used.

The conclusions of Brunton and Macfadyen,[1] that enzymes produced in meat broth liquefied gelatin more rapidly than did those produced in gelatin itself, would lend credence to the advisability of such a procedure.

Cultures started in this way quite uniformly liquefied one to two days earlier than did stab cultures made at the time that the broth cultures were mixed with the gelatin, showing that greater bacterial activity had resulted from the introduction of large numbers of bacteria into the gelatin, but the time required to prepare the "starter," together with the time required to obtain the liquefaction test in the enriched gelatin culture, was about the same as that required for a similar test with stab cultures direct.

A modification of the preceding method consisted in incubating a broth culture of the test organism for four days at 40° C. and then testing for the presence of liquefying enzymes by pouring the culture on the surface of gelatin after marking the level of the gelatin surface, determining whether liquefaction ensued by any change in the depth of the gelatin. This method proved to be less efficient as a means of rapid liquefaction diagnosis than was the preceding one.

Fermi[2] states that the production of gelatinase is more abundant in gelatin media than in any other media and his statement was confirmed by Abbott and Gildersleeve.[3] In the recent experiments of Jordan[4] it was found that the rate of enzyme production during the first 10 days with seven different rapidly liquefying species, was more

[1] *Proc. Roy. Soc.*, 1889, 46, p. 543.
[2] *Centralbl. f. Bakt.*, 1890, 7, p. 469.
[3] *Jour. Med. Res.*, 1903, 10, p. 42.
[4] *Biological Studies by Pupils of Wm. T. Sedgwick*, University of Chicago Press, 1906, p. 127.

rapid in broth than in gelatin at 37.5° C. in four out of seven experiments, while it was more rapid in broth at 20° C. in only two out of seven experiments.

Gelatin at 20° and 40° C.—The results obtained in the preceding experiments having shown that it was futile to attempt to obtain early liquefaction results by cultivation in broth, studies were commenced in which gelatin cultures were incubated at 40° C., the gelatin of course being fluid at that temperature. After some preliminary experiments, a routine procedure was adopted as follows: Cultures which had shown the power to liquefy gelatin at 20° C. during the regular *B. coli* tests, were transferred to agar streaks, grown over night at 40° C., and were then reinoculated into gelatin tubes and placed in the 40° C. incubator for four days. The fluid gelatin cultures so obtained were removed at the end of 24 hours, two days, three days, and four days, and placed on ice for one hour, after which treatment those tubes in which the gelatin had solidified were returned to the incubator and the cultures in tubes in which the gelatin failed to solidify in one hour on ice were recorded as having produced liquefaction.

The investigation extended over a period of 10 months ending November 1, 1903. During this time about 2,800 cultures obtained in routine *B. coli* determinations from samples from a variety of sources were submitted to the gelatin liquefaction test by the standard procedure, and 368, or 13 per cent of these cultures, were found to show signs of liquefaction in 14 days or less at 20° C.; 288 cultures, selected at random from among these liquefying cultures, were tested for liquefaction at 40° C. by the method described above. In the routine liquefaction tests at Lawrence the approximate time required for liquefaction to ensue is shown by readings made on the fourth, seventh, tenth, and fourteenth days, and the type of liquefaction has always been recorded, three primary types of liquefaction being recognized: the stratiform type, the funnel, including the saccate and infundibuliform types, and the cup, including the craterform and the napiform types. The detailed results of the tests at 40° C. are shown in Table 1, the cultures being grouped according to their variations at 20° C.

Of the 288 cultures tested by both methods liquefaction developed

TABLE 1.

RESULTS OF TESTS FOR LIQUEFACTION AT 20° C. AND AT 40° C.

GELATIN AT 20° C.			GELATIN AT 40° C.					
Type of Liquefaction	Date Liquefaction Was First Noted	Number of Cultures	Number of Cultures First Showing Liquefaction on				Total Number of Cultures	
			First Day	Second Day	Third Day	Fourth Day	Liq.	Non-Liq.
Cup.....	4th day	9	3	2	1	0	6	3
	7th	6	1	0	0	0	1	5
	10th	17	3	0	0	0	3	14
	14th	121	18	0	0	4	22	99
Funnel.....	4th	48	33	1	1	1	36	12
	7th	10	0	0	0	0	0	10
	10th	17	2	1	0	0	3	14
	14th	31	6	1	0	2	9	22
Stratiform.....	4th	21	12	3	1	0	16	5
	7th	3	1	0	0	0	1	2
	10th	2	1	0	1	0	2	0
	14th	3	0	0	0	0	0	3

at 20° C. in 27 per cent in four days or less, in 7 per cent between the fourth and seventh days, in 12 per cent between the seventh and tenth days, and in 54 per cent between the 10th and 14th days, 53 per cent of the liquefactions being of the cup type, 37 per cent of the funnel type, and 10 per cent of the stratiform type.

The rate of liquefaction at 20° C. by cultures producing the cup type of liquefaction was very different from that of cultures producing the funnel and stratiform types, 73 per cent of the stratiform liquefactions and 45 per cent of the funnel liquefactions being manifest within four days, while only 6 per cent of the cup liquefactions were noted within the same period. Furthermore, 79 per cent of the cup liquefactions, 29 per cent of the funnel liquefactions, and 10 per cent of the stratiform liquefactions were not manifest until after the 10th day. We thus observe that a shortening of the time of incubation of gelatin cultures to 10 days or less, as has been the practice in some laboratories, would result in the exclusion of four-fifths of the cup liquefiers, and from one-tenth to one-fourth of the other forms from among the liquefying types, with a consequent increase in the reported presence of *B. coli* in samples from which it was undoubtedly absent. The records of the examination of over 23,000 cultures obtained in routine *B. coli* determinations during the past five years, amply substantiate the statement that a shortening of the gelatin incubation period would introduce a serious error into the analytical results.

An examination of the results obtained at 40° C. reveals the fact that 28 per cent of all the cultures tested produced liquefaction within 24 hours, and 34 per cent within four days, while 66 per cent of the cultures failed to produce any signs of liquefaction whatever during the period of four days that they were under observation. A certain similarity between the behavior of cultures of the cup, funnel, and stratiform types at 20° and at 40° C. is also apparent; 65 per cent of the cultures of the stratiform type and 46 per cent of those of the funnel type showed liquefaction at 40° C. within four days, while only 21 per cent of the slowly liquefying cup type were able to produce liquefaction at the higher temperature within the same period. About four-fifths of all cultures which were able to produce peptonization at all during four days at 40° C. did so within the first 24 hours. The rate of liquefaction at 20° C. and at 40° C. by cultures of these different types is shown in the following table.

TABLE 2.

RELATIVE LIQUEFACTION AT 20° C. AND 40° C. BY CULTURES OF THE CUP, FUNNEL, AND STRATIFORM TYPES.

	All Cultures	Cup Type	Funnel Type	Stratiform Type
Number of cultures	288	153	106	29
Per cent. of cultures first showing liquefaction at 20° C. on the 4th day	27	6	45	73
Per cent. of cultures first showing liquefaction at 20° C. on the 7th "	7	4	10	10
Per cent. of cultures first showing liquefaction at 20° C. on the 10th "	12	11	16	7
Per cent. of cultures first showing liquefaction at 20° C. on the 14th "	54	79	29	10
Per cent of cultures first showing liquefaction at 40° C. on the 1st "	28	16	39	48
Per cent of cultures first showing liquefaction at 40° C. on the 2d "	3	1	3	10
Per cent of cultures first showing liquefaction at 40° C. on the 3d "	1	1	1	7
Per cent of cultures first showing liquefaction at 40° C. on the 4th "	2	3	3	0
Per cent of cultures liquefying at 40° C.	34	21	46	65
Per cent of cultures not liquefying at 40° C.	66	79	54	35

There is apparently very little correlation between the rate of liquefaction at 20° C. and liquefaction at 40° C. To be sure, a much greater proportion of the cultures which had produced liquefaction at 20° C. within four days reacted within a like period at 40° C. than was the case with cultures which produced gelatinase more slowly at the lower temperature. For example, 74 per cent of the four-day 20° C. cultures liquefied within four days at 40° C., while only 11 per cent, 22 per cent, and 20 per cent, respectively, of the 7-, 10-, and 14-day cultures reacted within the same period. That is to say, about one-fifth of the cultures which were unable to produce lique-

faction within one week at 20° had their peptonizing function so accelerated by incubation at 40° that they were able to react within four days. On the other hand, about one-fourth of the cultures which liquefied within four days at 20° C. were unable to produce a like reaction in the same period at 40° C., although taking all the cultures into consideration there was an increase of about 6 per cent in liquefactions recorded within four days at 40° C. over those recorded in the same period at 20° C. The failure of so many cultures to produce peptonizing enzymes at 40° C. in the same time that they were able to do so at 20° C. is especially significant, as it indicates that the optimum temperature for enzyme production is quite different with different cultures, and further suggests that if this be the case, it is quite possible that by cultivation at the proper temperature, the liquefying function might be induced in many cultures which have hitherto been classed among the non-liquefying types. Jordan[1] has recently demonstrated that the optimum temperature for the reaction between the liquefying enzyme and the gelatin and the optimum temperature for the bacterial production of that enzyme are often widely different. The relative liquefaction at 40° C. by cultures which produced peptonization in 4, 7, 10, and 14 days, respectively, is shown in the following table.

TABLE 3.

LIQUEFACTION AT 40° C. BY CULTURES LIQUEFYING IN 4, 7, 10, AND 14 DAYS RESPECTIVELY AT 20° C.

	Fourth Day	Seventh Day	Tenth Day	Fourteenth Day
Number of cultures	78	19	36	155
Per cent of cultures first showing liquefaction at 40° C. on the 1st day	61	11	18	15
Per cent of cultures first showing liquefaction at 40° C. on the 2d "	8	0	2	1
Per cent of cultures first showing liquefaction at 40° C. on the 3d "	4	0	2	0
Per cent of cultures first showing liquefaction at 40° C. on the 4th "	1	0	0	4
Per cent of cultures liquefying at 40° C.	74	11	22	20
Per cent of cultures not liquefying at 40° C.	26	89	78	80

CONCLUSIONS.

The results of the foregoing experiments have been almost completely a record of failures, due undoubtedly to the fact that the fundamental assumption, that since the growth of bacteria of the types in question was more rapid at 40° C. than at 20° C., the produc-

[1] *Loc. cit.*

tion of liquefying enzymes must also be accelerated at the higher temperature, was untenable. We have learned by past experience that the other biochemical functions by whose aid we are able to separate from the colon group many types of foreign bacteria, the production of gas and acid in sugar solutions, the reduction of nitrates, the coagulation of milk, and the production of indol are more active at the higher temperature. We learn from these experiments, however, that the liquefying function does not as a rule follow the same law as the other biochemical functions.

Concerning the attempts to prepare a gelatin medium which should be solid at a temperature of 40° C. upon which liquefaction might be noted in the same manner as that employed with ordinary culture gelatin at 20° C., it need only be said that, while such media can be made, its preparation is difficult, and by the use of more recent methods of testing for liquefaction, its use becomes unnecessary in dealing with the specific problem—the shortening of the time involved in making tests for *B. coli.*

The use of broth cultures to accelerate the production of liquefying enzymes also appears to be of doubtful value. From the comparative experiments with gelatin cultures at 20° C. and at 40° C. we see that more than half of the liquefying cultures commonly found during routine *B. coli* tests at Lawrence were of one type, with about four-fifths of which liquefaction was not manifest until after the 10th day, and with this type of cultures liquefaction was not produced within four days, when they were incubated at 40° C. Furthermore, we note that a very considerable proportion of cultures of the funnel and stratiform types, which usually produce liquefaction within four to seven days at 20° C., failed to show liquefaction within four days at 40° C. That is to say, while the activity of the liquefying function was greatly increased at the higher temperature in some instances, it was in other cases materially retarded.

The temperature at which the liquefying enzymes act most energetically upon gelatin has been determined by a number of investigators. The determination of the temperature at which the greatest bacterial activity in enzyme production shall be combined with the maximum liquefying activity of that enzyme appears, however, to have been overlooked, and it appears to the writer that data of this

character are necessary before we may formulate a method for determining liquefaction which shall be at the same time rapid and accurate. The preparation of media more favorable for rapid enzyme production than are the usual culture media, and the establishment of more refined chemical tests for the detection of minute quantities of the liquefying enzymes would also appear to be fertile fields for investigation.

THE USE OF LACTOSE-BILE MEDIUM IN WATER ANALYSIS.

DANIEL D. JACKSON.

(From Mt. Prospect Laboratory, Brooklyn, N. Y.)

THE determination of intestinal bacteria as represented by *Bacillus coli* gives, when properly interpreted, a most valuable index of the quality of a water from a sanitary standpoint. Unfortunately the presumptive test with Smith solution, owing to the interference of other growths of bacteria has often proved to be misleading, especially in badly polluted waters.

In a paper previously published,[1] entitled "A New Solution for the Presumptive Test for *Bacillus coli*," the author has described numerous experiments on the selective inhibiting effect upon the growth of bacteria by the various salts and their constituents found in the bile. By the use of bile media most species of bacteria other than *B. coli* are killed or restrained, thus allowing of the free action of the intestinal germs. The tests show that the cholic acid radical of the bile is the effective agent. This conclusion is important in that it admits of the use of sodium glycocholate as an inhibiting salt. In ox bile, which is the material most available, nearly all of the bile salt present is in the form of glycocholate.

The experiments also show that good results are obtained only when an amount of bile salt is used equal to that present in undiluted ox bile but that further concentration is unnecessary. Liquid ox bile filtered after sterilization contains about 110 grams of solid matter per liter, of which about 90 grams consist of bile salts. This is an amount far in excess of that previously employed as a restraining agent but a lesser amount gives less efficient results. The liquid also contains albuminous matter equal in food value to the meat extract employed in the usual media.

An undiluted bile solution sterilized when freshly drawn may be

[1] *Biological Studies by the Pupils of William Thompson Sedgwick*, University of Chicago Press, 1906.

kept in stock until ready for use. It is then decanted or filtered and 1 per cent of lactose, previously dissolved in a small amount of water, is thoroughly mixed with the bile, after which it is tubed and sterilized in an autoclave for 30 minutes at 15 pounds pressure. The fermentation tubes employed are 140 mm. long and 15 mm. in diameter, having an elongated bulb 38 mm. in its shortest diameter. This admits of the addition of 10 c.c. of the water to be tested without too great dilution of the medium.

A large number of market samples of evaporated or inspissated bile were tested, but they were all found to be very acid, and even when neutralized did not give proper results. It has since been established by experiment that if the bile is sterilized in an autoclave when freshly drawn and the filtered liquid evaporated to dryness that this product when kept perfectly dry will remain practically neutral and can be preserved indefinitely. A combination of 110 grams of the solid bile and 10 grams of lactose dissolved in a liter of distilled water will give the same results as the medium made from fresh liquid bile.

It has been shown by extensive tables given in the article previously referred to that negative results for *B. coli* have been often found by the use of Smith solution when there was no question that the germ was present but had been overgrown in the medium and crowded out by other bacteria. On the other hand, experiments recently performed show that apparent positive tests for *B. coli* may be obtained when a mixture of gas producing bacilli other than *B. coli* are added to Smith solution.

The results so far obtained with bile lactose medium point strongly to the fact that no gas producer or mixture of gas producing bacteria will give results as high as 25 per cent of gas except *B. coli*, even when three days' incubation is employed. Attenuated *B. coli* sometimes requires three days for its development to that point. The necessity for the absorption test is therefore eliminated.

In testing a water for *B. coli* dilutions of 0.1, 1.0, and 10 c.c. are planted in the fermentation tubes and incubated at 37.5° C for 48 hours. All tubes giving over 25 per cent of gas are considered to contain *B. coli*. Those having any considerable amount of gas but still under 25 per cent are returned to the incubator for 24 hours longer.

Attenuated *B. coli*, as before stated, will sometimes require 72 hours to give the positive test.

Underground waters not exposed to surface influences and properly filtered waters should not give positive tests in any dilution. Surface springs, open dug wells, and pure surface waters may give positive tests in the 10 c.c. dilution, but in no less quantities. Moderately contaminated waters and those exposed to surface drainage containing small quantities of the excreta of the lower warm-blooded animals may give positive tests in the 1 and in the 10 c.c. dilutions. Such waters are to be looked upon with suspicion. Contaminated waters and those having a large amount of surface wash will give positive tests in the $\frac{1}{10}$, 1, and 10 c.c. dilutions. For grossly polluted waters and sewages higher dilutions may be employed, as in testing sewages and sewage effluents from filters.

At Mt. Prospect Laboratory about 5,000 samples of waters of various degrees of purity and from hundreds of different sources have been tested by means of lactose-bile media, and the results have always agreed with the judgments formed by the complete analysis of the water as well as by careful sanitary inspections of the individual sources of supply. In many instances contamination has been found to exist which would not otherwise have been detected.

The use of this medium gives an exact gauge of the purification brought about by systems of filtration, and in the New York City, Department of Water Supply, Gas and Electricity; six larges and filter beds and two mechanical filter plants, under the author's charge in the Borough of Brooklyn, are daily tested by this method as well as for quantitative bacterial removal.

CONCLUSIONS.—The bile medium previously employed has contained too little bile salt to be effective as a selective inhibiting agent.

The use of the lactose-bile medium herein described for the determination of *B. coli* prevents negative tests from the overgrowth of other bacteria as well as positive tests from mixtures of other gas formers.

By the employment of this new medium more definite results on the sanitary quality of a water may be obtained than by any other test at present in use. The medium is also especially valuable in the testing of filter plants.

EXPERIENCE WITH LACTOSE-BILE MEDIUM FOR THE DETECTION OF *B. COLI* IN WATER.

LUTHER R. SAWIN.

(From Mt. Kisco Laboratory, Mt. Kisco, N. Y.)

THE discovery of *B. coli* in 1885 by Escherich, coupled with the knowledge that it is a normal inhabitant of the intestine of man and many animals, and therefore is found in sewage and polluted water, has induced water-bacteriologists to pay more attention to its isolation than to any other part of their work. Various methods have been proposed and tried for the detection of this organism in water. The one considered most reliable and meeting with general approval has involved the planting of a portion of the sample, either directly or after revivifying in broth, into plates of lactose-litmus agar; and then putting those colonies developing *B. coli* characteristics through the different tests as recommended for the identification of this organism. This procedure although accurate, is at the best a slow, tedious, and laborious process. Moreover, it is impracticable when used by the supervisor of a public water-supply, whose duty it is to report on the daily sanitary condition of the water. For example, if two days are required for the water to reach the inhabitants of the city, and it takes seven days to learn the results by this process, the consumer will have used the water five days before he is informed of the danger.

To obviate these difficulties, various rapid methods have been proposed and used by different water-analysists. Nearly all are based on the fact that *B. coli*, when grown in a fermentable sugar, generates a certain amount of gas of which a definite proportion is carbon dioxid. In many cases certain chemicals whose function it is to inhibit the growth of the water bacteria have been added to the sugar solution. The most common is phenol, either alone or in combination with hydrochloric acid. McConkey has recommended the addition of sodium taurocholate to agar; others have used this compound in Smith solution. It would seem that these different methods, all based on the same general principles, ought to give something like concordant results. But unfortunately this is not the case and they

do not check one another even within reasonable limits. The use of phenol has called forth considerable controversy as to its merits. In this laboratory, where it has been used more or less in an experimental way, it is the experience of the author, that when used to test sewage or badly polluted waters, it gives very satisfactory results, but when used to test a questionable water or one containing few *B. coli* or *B. coli* in an attenuated condition, it generally fails to give the positive test. In a case like this Smith solution alone is preferable. It is very evident that a method requiring a previous knowledge of the character of the water for its use is of very little value.

On the other hand, many bacteriologists, recognizing the uncertain value of phenol in media, prefer to use Smith solution without the addition of any reagent having an inhibitory effect. With the use of Smith solution the conditions are just reversed. And in this laboratory, where it has been used since its establishment in 1903, it has been found very satisfactory with good or questionable waters, but with sewage of badly polluted streams, it often completely fails. Frequently with sewages, the fermentation tubes show no gas production whatever, even when inoculated with very small quantities of the sewage.

As a solution of the problem attending the sanitary examination of water, Mr. Daniel D. Jackson,[1] of Mt. Prospect Laboratory, Brooklyn, N. Y., has proposed the use of lactose-bile medium for the fermentation test. This medium consists of undiluted liquid ox bile sterilized when freshly drawn and containing 10 grams of lactose to the liter of bile. The medium has been used in this laboratory for the last five months in conjunction with the presumptive test. In all 1,076 samples have been examined by both methods, and, as far as possible, waters of a varied character have been used ranging from sewages to waters whose purity is beyond question. Not only have comparisons been made as to the relative value of the two methods, but waters, whose sanitary condition is known by actual inspection of the local conditions have been examined with lactose-bile in view of ascertaining how far the results conform to the character of the water.

Aside from the fact that the presumptive test is unreliable in the examination of polluted waters, there is another factor which tends

[1] *Biological Studies by the Pupils of William Thompson Sedgwick*, University of Chicago Press, 1906.

to throw discredit on its results. Reference is now made to the large number of anomalies which this method furnishes. For example: if fermentation tubes of Smith solution are inoculated with 0.1, 1.0, and 10.0 c.c. portions of the sample, frequently a positive test is obtained in the 0.1 but not in the 1.0 and 10.0 c.c. tubes. And, what is still more puzzling, occasionally, the sample gives a test in the 0.1 and 10.0 c.c. inoculations, but not in the 1.0 c.c. With bile medium, while occasional anomalies have been found, there has been a great reduction, as may be seen from the following data: Of 1,063 samples, duplicate analyses were made in lactose-bile and Smith solution. In the 1.0 c.c. portions 2.73 per cent gave anomalies by the former and 8.00 per cent by the latter method. With the 10.0 c.c. portions, the bile medium gave 4.73 per cent against 13.73 per cent anomalies by Smith. In both cases, the reduction in anomalies was approximately two-thirds.

With the use of the presumptive test for *B. coli*, it has been the practice to consider the test positive, when the total gas production was 25–70 per cent of total volume, and the carbon dioxid 25–40 per cent of this amount. Whipple, Irons, and other authorities agree that the percentage of carbon dioxid should be about 33 per cent of total gas evolved. Experiments in this laboratory, with pure cultures of *B. coli* in fermentation tubes of Smith solution and lactose-bile show that with the former there is a wide range in the percentages of carbon dioxid found. Nineteen inoculations in Smith solution gave an average of 27 per cent carbon dioxid. With the lactose-bile, the results obtained were more uniform, and the average percentage of carbon dioxid obtained was appreciably higher, being 37 per cent. In actual experience, with tests on polluted streams, it has been found that the percentage of carbon dioxid as found by lactose-bile medium has been about 39 against 30 with Smith. Table 1 illustrates this point very clearly.

In this table, the 1–1,000 and 1–100 dilutions are samples of sewages and street wash from which *B. coli* have been repeatedly isolated. As other examples to illustrate the differences in the percentages of carbon dioxid as found by the two methods, tests were made on two badly polluted streams. The first receives sink drainage and street wash; the second, barnyard drainage and street wash. Twenty

TABLE 1.

Dilution	Smith Solution		Lactose-Bile Solution	
	No. Sample Examined	Per Cent CO_2	No. Samples Examined	Per Cent CO.
1–1,000	9	30.00	14	39.17
1–100	14	27.40	26	39.62
1–10	352	30.51	214	39.93
1	526	29.29	430	39.65
10	649	29.71	618	38.89

samples from each stream were analyzed, and with the first, the average percentage of carbon dioxid (including 0.1, 1.0, and 10.0 c.c. portions) was 29.24 by Smith against 40.09 by lactose-bile. With the second stream, averages of 28.96 and 39.20 per cent carbon dioxid were found by tests with Smith and lactose-bile solutions, respectively.

In the second portion of this paper it is the object, first, to show the relative value of the two methods when used on different classes of waters; and second, and more important, to show how the results obtained by the lactose-bile method compare with the known facts ascertained by inspection of the local conditions in which the waters under consideration are found.

For this purpose, series representing polluted and unpolluted waters have been selected and arranged according to their sanitary qualities as shown in Table 2, and the average percentage of samples giving positive tests for *B. coli* with Smith and lactose-bile solutions recorded in the subsequent columns. For the wells, these figures are an average of 50 analyses; for the surface waters and sewage, an average of about 20 analyses. Samples numbered 1 are waters from deep, driven wells not open at the top; those numbered 2 are waters from shallow, open, dug wells. Waters numbered 3, 4, 5, 6, 7, and 8 are from lakes situated in sparsely inhabited regions where there is little danger of pollution. The next group of waters (Nos. 9. 10, and 11) are more or less polluted. No. 12 is a badly polluted stream; No. 13 principally a mixture of street wash and household drainage; No. 14 is a septic sewage.

An examination of Table 2 shows that, with the first eight waters, there is very little difference in the figures obtained by either method. With well waters, it is extremely rare that *B. coli* is found in

the 10 c.c., and it has never been found in the 0.1 and 1.0 c.c. portions although large numbers of these waters have been examined with lactose-bile medium. With the waters from the lakes, which are situated in sparsely inhabited regions, the small amounts of coli are due, no doubt, to the effect of small animals. The results in all these samples indicate waters of an excellent quality.

With the next three waters, which are more or less polluted, the percentages of positive tests with the bile medium are appreciably higher than those found with the Smith solution. The results with the lactose-bile would seem to be more in accordance with the facts. With this group of waters, at times *B. coli* can be found and at others it cannot be detected with Wurtz agar.

If, from the preceeding results, there is any question as to the relative value of the two methods, or as to the value of the lactose-bile method when used for the detection of *B. coli* in polluted waters, it is removed after examining the results which samples Nos. 12, 13, and 14 furnish. In every case analyses by the lactose-bile method gave decidedly higher results than by the Smith; and in the 1.0 and 10.0 c.c. portions the percentages of positive tests were close to 100. With the sewage, while there was an occasional negative result, about 90 per cent of the tests were positive with the bile against only 25 per cent with the Smith. With the three samples, now under

TABLE 2.

No.	Source	Percentage of Samples Giving Positive Smith Solution			Tests for *B. coli* by Lactose-Bile Medium		
		0.1 c.c.	1.0 c.c.	10.0 c.c.	0.1 c.c.	1.0 c.c.	10.0 c.c.
1	Deep wells	0.	0.	0.	0.	0.	0.
2	Shallow wells	0.	1.0	10.0	0.	0.	6.0
3	Lake	15.0	10.0	15.0	5.0	0.	15.0
4	Lake	5.2	15.7	21.0	5.2	5.2	31.0
5	Lake	10.0	5.0	40.0	10.0	5.0	15.0
6	Lake	10.0	10.5	26.0	10.0	10.5	50.0
7	Lake	0.	10.0	5.0	00.	0.0	15.0
8	Lake	10.0	15.0	35.0	0.	5.0	30.0
	Average, Nos. 3, 4, 5, 6, 7, and 8	8.3	11.0	23.6	5.0	4.3	26.0
9	River	47.0	72.2	55.5	50.0	75.0	84.2
10	River	26.3	37.6	73.7	30.0	90.0	85.0
11	River	36.8	55.1	68.9	40.0	73.5	78.1
	Average, Nos. 9, 10, 11	36.7	55.1	66.0	40.0	79.4	82.4
12	Brook	47.7	63.2	72.2	60.0	90.0	84.2
13	Drainage	50.0	73.7	78.9	84.2	90.0	90.0
14	Sewage	25.0	25.0	8.2	87.5	93.7	81.2
	Average, Nos. 12, 13, 14	40.9	53.9	53.1	77.2	91.2	85.1

consideration, the averages of the positive tests obtained by the two methods show that the bile gave nearly twice as many positive tests as the Smith. These three samples give results corresponding very closely to what one would expect and what one should find in the examination of polluted waters.

SUMMARY.

The presumptive test, using lactose-bile medium, is a rapid and practical method for the detection of *B. coli*. If the absorptions are omitted, as the data obtained from experiments seem to show that they may be without detracting from the value of the test, the routine work is generally lessened.

The anomalies obtained by this method are few and much less than when Smith solution is employed.

The percentage of carbon dioxid is decidedly higher when lactose-bile is used for testing waters than when Smith solution is employed. This fact corresponds with the results obtained by experiments with pure cultures of *B. coli* in lactose-bile and Smith solutions.

Both methods give substantially the same results when used for testing "safe" waters, and the results are consistent with the facts learned by observation. With questionable or contaminated waters, the percentage of positive tests obtained with the use of lactose-bile media are appreciably higher than those obtained with the use of Smith solution. With sewages or badly polluted waters, lactose-bile medium gives a decidedly greater number of positive tests than the Smith solution.

For general convenience in the routine work of a laboratory, for practicability in the examination of public water-supplies, and for reliability in testing waters of a varied character, the fermentation test, using lactose-bile medium, is the most promising method that has yet been advanced.

COMPARATIVE RESULTS OBTAINED BY THE USE OF LACTOSE-BILE AND DEXTROSE-BROTH MEDIA FOR THE DETECTION OF *B. COLI* IN WATER.

ROBERT SPURR WESTON AND RALPH E. TARBETT

FOR some little time past comparative tests have been made with the use of dextrose-broth and lactose-bile media in the preliminary enrichment tubes in the examination for *B. coli* in the raw and filtered water of the Tennessee River at the Laboratory of the Knoxville Water Company, Knoxville, Tennessee.

The lactose bile has been prepared from fresh local ox-bile to which 1 per cent of lactose has been added just before sterilization. The dextrose-broth has been prepared according to the directions given in the "Standard Methods of Water Analysis."[1]

The method used in the isolation of *B. coli* has been that prescribed by the Standard Methods. We have not, however, attempted to rejuvenate, by successive enrichment, debilitated forms which have lost some of their powers to react with the various media.

In recording results failure to give the correct gas ratio with dextrose-broth, or failure to give the indol reaction with the peptone solution, have been classed as atypical, while cultures which fail owing to the liquefaction of gelatin have been classed as belonging to the *B. cloacae* group. We have made comparative results with 63 samples of water. Of this number, 21 gave no gas formation with either media, 18 gave gas formation with both media, and 24 gave gas formation with dextrose-broth, but not with lactose-bile.

It has been our observation that the formation of gas takes place much less rapidly in the lactose-bile than in the dextrose-broth medium, there being a comparatively small amount of gas formed in the first 24 hours. In some cases more than 24 hours were required before the appearance of gas was noted in the lactose-bile solution. In no case did the amount of gas formed in 48 hours exceed 65 per cent of the closed arm of the tube, while the average amount was

[1] *Jour. Infect. Dis.*, Supplm. 1, 1905, p. 1.

43 per cent. The ratio of CO_2 to the total gas in the tubes containing lactose bile varied very much as the ratio varies in the case of dextrose broth.

The isolation results obtained from the preliminary cultivation tubes of the 18 samples which gave gas formation with both dextrose-broth and lactose-bile are as follows:

No. Samples	Dextrose-Broth	Lactose-Bile
4	Typical *B. coli*	Typical *B. coli*
1	Typical *B. coli*	Atypical form
1	Streptococci	Typical *B. coli*
3	Atypical forms	Atypical forms
1	Streptococci	Atypical form
4	*B. cloacae*	Atypical forms
4	*B. cloacae*	*B. cloacae*

It is seen that the results of the isolation from the lactose-bile enrichment were with four exceptions either the typical *B. coli* or very closely related forms. The microscopical examination of the cultures from the lactose-bile enrichment tubes showed pure cultures whose physical characteristics agreed with those of *B. coli.*

It is possible that, in those cases where atypical results were obtained, positive results might have been obtained by further rejuvenation in nutrient broth.

Of the 24 samples showing gas formation with dextrose-broth but not with lactose-bile, the following results were obtained:

No. Samples	Dextrose-Broth
1	Typical *B. coli*
7	Atypical forms
2	Streptococci
2	*B. cloacae*
9	Failure on litmus lactose agar
3	Negative forms not classified above

We make no comments on the results, but simply state the facts as observed thus far by us in the comparative use of the two media.

SANITARY CHEMICAL EXAMINATION OF WATER BACTERIA.[1]

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IN view of the modern theory of disease the problem of sanitary water analysis concentrates itself on the bacteriological rather than on the chemical condition of the water in question, whatever the method may be of arriving at this knowledge. All waters would be perfectly safe for drinking purposes, with certain well-characterized exceptions in the case of mineral constituents, if only the pathogenic bacteria were removed. Our investigations are, therefore, directed toward those bacteria that are pathogenic to man, are capable of being borne by water, and have the possibility of infecting the human subject through the alimentary tract.

The routine sanitary chemical tests, all of which are for substances perfectly harmless in themselves, are merely an indirect method of determining the bacteriological condition of the water. Naturally with such an indirect method there is a very wide range of possibilities in the interpretation of the results. The analytical data of a sanitary chemical analysis indicate merely the presence, recent presence, or possible future presence of pathogenic bacteria. It follows accordingly that by current methods the sanitary chemical analysis of pure distilled water to which a little sterile bouillon had been added would indicate a dangerously polluted water.

Naturally, with the advancement of bacteriology, more direct methods of analysis have been undertaken. These are of course limited to quantitative and qualitative investigations of the bacteria. One does not need to emphasize the difficulty of establishing a standard for the maximum number of bacteria permissible in a potable water, or of the uselessness of searching the Mississippi River or the Great Lakes for a typhoid bacillus.

[1] This work was carried on under co-operative agreement between the Illinois State Water Survey, State Geological Survey, Engineering Experiment Station of the University of Illinois, and the Division of Hydro-Economics of the United States Geological Survey.

The only qualitative work attempted at present is the identification of intestinal forms. The presumptive coli tests are in a very uncertain and unsatisfactory condition. The quantitative estimation of coli is well-nigh impossible in a laboratory where a large number of samples are received for daily routine examination. Though possible presence or absence can be quite satisfactorily made out, even when many samples require attention at the same time, an opinion cannot be based on a qualitative test alone. It is essential to know the quantity of coli bacteria present.

Of the various routine methods of procedure, the direct chemical analysis, being the broadest of all, has one advantage over all, or one disadvantage as the case may be. Such an analysis does not depend on the presence of living active organisms as do all bacterial methods; but the pollution may still be detected where bacteria have died in large quantities after exhausting their food material. Also the chemical analysis will detect the pollution where soil-filtered sewage reaches a water-supply. Probably the most necessary factor in interpreting the sanitary chemical analysis is a thorough knowledge of the source of the water under examination. In this section of Illinois one is utterly helpless in the interpretation of the analysis of a well water, unless he knows whether the well is deep or shallow and in drift or in rock.

The results obtained by analyzing the water from two wells in Urbana will illustrate the difficulty. Amounts are stated in parts per million.

	No. 1	No. 2
Total residue on evaporation	415.	365.
Chlorine in chlorides	3.5	7.5
Oxygen consumed	5.2	2.0
Nitrogen as free ammonia	3.6	0.054
Nitrogen as albuminoid ammonia	0.136	0.056
Nitrogen as nitrites	0.040	0.030
Alkalinity	353.	245.
	1 c.c.	1 c.c.
Bacteria at 20° C.	200.	1000.
B. coli communis	Absent	Present

No. 1 is from a well 180 feet deep and has high oxygen consumed and high free and albuminoid ammonia, which are characteristic of deep wells in the drift in central Illinois. Bacterial tests show that the water is in good condition.

No. 2 is a water from a 30-foot dug well, and chemically, according to usual methods of interpretation, it is a better water than No. 1. Bacterially it contains 1,000 bacteria per c.c., and the reaction is positive to the presumptive test for coli.

Generally speaking, the chemist must know the normal constituents of a given locality before he can determine the amount due to pollution.

In addition to the sanitary chemical analysis and the quantitative and qualitative bacteriological examination, a further line of procedure is suggested to the scientist, that is, the chemical analysis of bacterial cultures obtained by the inoculation of water samples into artificial media.

Our experiments upon this principle are based on the supposition that, by the inoculation of water into artificial sterile media, we could, in a way, imitate the changes that would ordinarily be brought about by bacteria in a water containing natural media. One great advantage would lie in the ease with which the artificial media could be analyzed, and the accuracy with which the changes due to the water could be determined. By this means it is possible that local factors which affect the interpretation of the results of analysis may be removed. There arises the possibility of establishing an absolute standard for the maximum limits of impurities.

To establish the value from the sanitary standpoint of the analysis of the cultures of water, our experiments are carried out with a view to securing constancy of results. After a few preliminary tests (see Table 1) we chose for a medium an ordinary meat-extract broth of double concentration, to which 2 per cent of gelatin was added. The presence of sugars prevented decomposition of the nitrogenous constituents. When it was desired to study the nitrogenous decomposition products, sugars were not added to the medium, although the extremely small amount of sugar present in ordinary acid meat extract was not removed.

For our first experiments we chose two types of water, one from a small creek, known to be seriously polluted, the other from deep, driven wells of unquestionable purity. We measured accurately 5 c.c. of each medium into test-tubes, inoculated with 1 c.c. of each sample of water, and incubated at 37° C. In these preliminary tests, the cultures, when they showed appreciable differences, were steamed in an Arnold sterilizer 20 minutes, diluted to 1,000 c.c., and subjected to sanitary analysis. *A priori* it was expected that it would be necessary to work with very young cultures, presuming that for old cultures the ultimate analysis of a pure and of a polluted water would be very similar. The waters tested thus far have given decidedly the contrary result. Table 1 is characteristic of the effect of

variations in media and age of culture. The results are expressed in parts per million of the diluted culture, 5 c.c. of the inoculated medium diluted to 1,000 c.c. with pure distilled water.

TABLE 1.

COMPARISONS OF THE ACTION OF PURE AND POLLUTED WATER ON MEDIA OF VARIOUS COMPOSITIONS FOR DIFFERENT PERIODS OF TIME.

SAMPLE	OXYGEN CONSUMED		ACIDITY		AMMONIA				TOTAL SOLIDS		NITRATES		CHLORIDES	MEDIA
					FREE		ALBUMINOID							
Hours...	24 hrs.	89 hrs.	24 hrs.	89 hrs.	24 hrs.	89 hrs.	24 hrs.	89 hrs.	24 hrs.	89 hrs.	24 hrs.	89 hrs.	89 hrs.	
Tap.....	102	75	12.13	12.13	0.64	1.12	8.00	13.6	...	212	0.48	0.40	37.0	
Creek....	90	55	12.13	21.82	4.60	13.20	8.00	4.8	...	124	0.40	0.44	35.5	Broth
Control..		75		7.27		0.64		6.80		254		0.48	37.5	
Tap.....	165	170	16.97	15.52	0.72	1.00	8.80	14.	...	386	....	0.40	36.0	
Creek....	145	145	36.37	54.80	0.76	1.00	7.60	12.0	...	309	....	0.72	36.0	Glucose
Control..		165		14.55		0.64		6.4		433		0.52	35.0	Broth
Tap.....	94	92	14.55	19.40	0.52	1.04	18.80	23.2	428	308	....	.56	34.5	
Creek....	104	60	24.25	67.90	3.60	23.20	18.80	10.4	397	168	....	0.64	32.5	Gelatin
Control..		85		9.70		0.64		13.2		359		0.52	32.5	Broth
Tap.....	185	183	21.82	26.67	0.76	1.12	18.00	23.20	612	489	0.72	0.48	36.5	Gelatin
Creek....	195	163	33.95	60.62	0.84	1.36	19.20	22.4	574	400	0.80	0.60	36.0	Glucose
Control..		180		16.97		0.64		13.60		557		0.44	37.0	Broth

As the most striking changes took place in the nitrogenous constituents, we decided to secure a series of preliminary tests determining only free ammonia. We were sometimes able to nesslerize directly, though more frequently, on direct nesslerization, we secured a greenish color, not comparable with a true nessler color.

In an attempt to establish a maximum limit of free ammonia, allowable, we obtained samples from deep wells of unquestionable purity, from the best available shallow wells, and from chemically pure water artificially polluted. In the following table the cultures were analyzed at different ages as a sufficient variety of waters had not yet been analyzed to determine the most favorable age of culture.

In Table 2 the following facts are of special interest. The previous statement that the chemical analysis of pure, deep, and shallow ground waters is entirely different is verified. Noticeable examples are Nos. 18 and 19. In No. 18 and No. 19 the results of the bacteriological analysis are practically the same and also the chemical analysis of the cultures of the two agree very well. This strengthens our hope that a universal standard of purity may be established.

TABLE 2.

COMPARISON OF SANITARY ANALYSES OF WATERS, FROM VARIOUS SOURCES, WITH THE AMMONIA THAT IS OBTAINED FROM MEDIA BY INOCULATION

Sample			Bacterial Analysis		Chemical Analysis, Parts per Million								Analysis of Culture, P. M. 1–200 Dil.		
					Nitrogen as								Ammonia		
No.	Source	Lab. No.	No. per c.c.	Coli in one c.c.	Free	Albuminoid	Nitrites	Nitrates	Total Solids	Oxygen Consumed	Alkalinity	Chlorides	Free	Alb.	Age Cult. in hrs.
1	Tap, deep, driven wells		200	–	3.6	0.136	0.004	0.04	415	5.2	353	3.5	0.36	....	24
2	"Boneyard" polluted creek		17,000	+	2.8	0.64	0.015	0.585	325	10.75	374.4	45.0	5.80	....	24
	Control												1.00	....	..
3	Tap, deep, driven wells	Same	as No. 1	(above)									0.12	....	16
4	Driven wells	Same	as No. 1	(above)									0.56	12.0	69
5	"Boneyard"	Same	as No. 2	(above)									1.52	....	16
6	"Boneyard"	Same	as No. 2	(above)									18.00	5.2	69
	Control		14,790										1.20	0.8	..
7	Vermillion River, raw	14725	2,600	+	0.120	0.336	000	0.40	359	7.15	234.7	3.5	29.00	....	68
8	Vermillion River, filtered	14734	120	?+	0.024	0.144	0.01	0.32	322	4.65	215.3	4.0	1.76	....	68
	Control		14,791										0.64	....	..
9	Country well, 30 feet deep	14827	300	+	0.020	0.094	0.004	0.32	608	.....	302.6	16.0	4.8	....	63½
10	Tap, deep, driven wells	Same	as No. 1	(above)									0.2	....	63½
	Control												1.2	....	..
11	Shallow well, 20 feet	14978	700	+	0.014	0.048	0.012	0.548	572	2.25	253.4	22.5	1.4	....	48
12	Shallow well, 30 feet	14979	1,000	+	.054	.056	.030	0.190	365	2.00	245.7	7.5	2.4	....	48
13	Deep well, driven	14980	120	–	1.768	.016	.000	0.040	384	2.75	353.2	2.5	0.16	....	48
14	Shallow well, 30 feet	14981	120	–	0.240	0.128	0.125	7.08	384	.00	234.2	7.5	0.16	....	48
15	Shallow well, 40 feet	14984	10	–	.082	0.64	000	0.040	529	1.50	316.8	21.0	0.08	....	48
16	Driven well	14986	19	–	.032	.056	000	.028	475	1.7	291.	14.0	0.00	....	48
17	Driven well, 30 feet	14987	33	–	.088	.082	000	0.40	561	2.75	251.	13.5	0.20	....	48
18	Driven well, 125 feet	14988	55	–	3.60	0.184	000	0.04	439	6.4	350.	3.5	0.00	....	48
19	Shallow well, 18 feet	14990	62	–	0.03	0.094	000	0.32	342	1.65	301.	6.5	0.08	....	48
20	"Boneyard" polluted creek	14992	55,000	+	2.8	0.64	.015	.585	325	10.75	374.4	45.0	9.40	....	48
	Maximum for this locality for shallow well		500	–	0.02	0.05	0.001	5.0	500	2.0	.....	15.0	....	....	..
21	Sewage 1–1,000 dilution		1,500	+	0.032	0.016	000	0.010	.....	0.6	.....	.05	1.72	....	48
22	Sewage 1–10,000 dilution		150	–	0.018	0.010	000	00	.....	0.3	.....	00	0.20	....	48

Analyses No. 7 and No. 8, raw and filtered river waters, furnish another good example to support our theory. On the whole, direct chemical analyses do not show the true efficiency of a water-filtration plant, as the results approximate each other rather closely. The bacteriological analyses show decided differences, about 97 per cent of the bacteria having been removed by filtration. The chemical condition of the two cultures varies widely. Ordinarily this would be adding comparatively little to the results of colony counts which show the bacterial efficiency of the filter; but in this case, although the efficiency of the filter was 95.4 per cent (there being only 120 bacteria per c.c. in the filtered product) the water responded to presumptive coli tests. After a long series of cultures no typical coli communis could be isolated, while the culture analysis was obtained on the third day with comparatively little effort. Even this time was unnecessarily long. It seems to us that the relation of this test to the presumptive coli tests will bear further investigation.

The analysis of cultures may also show qualitative as well as quantitative differences in water as indicated in samples No. 9 and No. 10. Here the chemical analyses are comparable and the number of bacteria practically the same. There is a difference in the quality of the bacteria as shown by the presumptive coli test and this difference shows up distinctly upon analyzing the culture. No. 9, which gave the positive coli test, gave 4.8 parts of free ammonia to the culture, while No. 10, which gave the negative test for coli, gave only 0.2 parts of free ammonia to the culture.

Realizing the importance of nitrite determinations in water analysis, we undertook to study their action. As indicated in our first table, the action of nitrites and nitrates could best be studied in media to which nitrites and nitrates had been added. We accordingly added 0.05 per cent sodium nitrite ($NaNO_2$) to part of our ordinary media. The results of a single experiment were as follows:

NITRITE MEDIA CULTURES.

Tap water gave	7.5	parts per million N as nitrite.
Boneyard gave	0.0	" " " " " "
Media Control gave	7.5	" " " " " "

The above test, we believe, differentiated very sharply in this instance, between the nitrites formed by putrefactive bacteria and the nitrites normally very high in a pure water.

According to present methods of interpretation a good water may be condemned on high nitrites alone; but the absence of nitrites does not show that a water is good. Further, the presence of nitrites in shallow wells is assumed to be due not to normal constituents of the soil, but to bacterial action. It is still further assumed that the bacteria concerned are putrefactive forms. Possibly the extent of the assumption is not thoroughly understood. Deep ground waters often contain high nitrites, their presence being explained by the supposition of the reduction of the nitrates present by the ferrous iron. We have, however, isolated very active denitrifying bacteria from these waters, and also from the air. Such bacteria might very naturally gain access to shallow wells containing nitrates and form nitrites. As compared with the relatively few denitrifying bacteria, there is the great mass of nitrifying bacteria universally present in the soil and upon which the preservation of life upon the face of the earth depends. When we consider these two great classes of bacteria it does not seem to us to be proven that the presence of nitrites should condemn a surface water.

The sanitary chemical tests, developed as they were on an entirely empirical basis, before the science of bacteriology was scarcely begun, were naturally difficult of interpretation and mistakes were unavoidable.[1] Our experiments indicate that the present interpretation of nitrites is partly in error, in that high nitrites could not normally be present in a shallow well.

Except in extreme cases it is often impossible to give an opinion of an Illinois water from the ordinary sanitary data, chemical and bacteriological, simply on account of the lack of evidence furnished concerning the source of the sample. Other authors have had the same experience. M. O. Leighton[2] says:

> There has been in the past surprisingly little discrimination used with reference to the selection of determinations for specific purposes. . . . . If . . . . it is desired to determine the amount of organic pollution in a water and show its value for domestic use, the chemist forthwith begins his round of nitrogen determinations, and closes with a statement of the oxygen consumed and the number of bacteria per c.c. In only a few well-known laboratories has this rule been violated, and such is the conservatism in the chemical profession that it will probably be largely followed in

[1] W. P. Mason, *Examination of Water*, 2d ed., New York; criticism of Wanklyn.

[2] T. M. Prudden, *Drinking-Water and Ice Supplies*, 2d ed., New York, 1901.
Field Assay of Water, Water Supply and Irrigation Paper, No. 151, pp. 10 and 11.

the future. . . . . The occasional isolated sanitary analysis of a water is positively without value. There are throughout the country numerous state, municipal, and private laboratories in which sanitary analyses are carried on. The water analyzed may be today from a well, tomorrow from a brook, and the next day from a pond. From the results of a single analysis wise and ponderous verdicts are sent broadcast, and the eager, waiting public is duly impressed.

As the basis of our experiments we have taken up the interpretation of chemical data from a theoretical standpoint, and we believe that this standpoint is absolutely essential to the intelligent understanding of sanitary data. For example, oxygen consumed, nitrites, nitrates, total solids, albuminoid ammonia, and chlorides, when *high*, indicate putrefactive bacteria. Putrefactive bacteria under proper conditions *reduce*, often very strikingly, the oxygen consumed, nitrites, nitrates, total solids, and albuminoid ammonia. These statements are in perfect harmony. (Septic tank data would confirm this.) Thus as the organic matter is converted to inorganic matter, the oxygen consumed (organic) decreases as the free ammonia (inorganic) increases.

The culture tests, since they discriminate sharply between putrefactive changes and normal variations, seem especially adapted for water analysis. The specific tests for sanitary purposes, which seem to be especially applicable, are free ammonia and nitrites.

In its present stage of development we recognize that, as in all other methods, no distinction is made between pollution from animal and human sewage. At times this distinction may be of paramount importance when we consider that typhoid bacteria are neither harbored by lower animals nor multiply in natural water.

We realize that the foregoing experiments can be regarded only as preliminary. Before absolute conclusions can be drawn more experimental work must be done. We would suggest the following topics:

Experiments to illustrate the action of pure cultures of water-borne bacteria on media of various composition.

Experiments to determine the proportion of cultural changes due to the bacteria and to the chemical condition of the inoculated water samples.

Experiments with media of different composition, with especial reference to acidity, and nitrogen, sulphur, and carbohydrate compounds.

Study of both the (1) formation of putrefactive products and (2) their fate when added to the original media; e. g., the formation of nitrites from nitrite-free media rich in nitrates or saline ammonia and their removal when added directly to the original

media, or the formation of free ammonia from proteids or its reactions when added to the original media.

Further comparisons of water known to be pure and of water known to be polluted, and artifical pollutions with sewages and pure cultures, guarding against any errors that might arise from overgrowths.

Experiments with cultures of different ages and temperatures.

Experiments on the possibility of mailing small un-iced samples. The forms ordinarily multiplying under these conditions are not the ones which develop at 37°.[1]

Put pure cultures in water, dilute and incubate with different dilutions.

It is our hope that other investigators in the field of water analysis may take up with us the study along the lines suggested. In closely related work on the bio-chemistry of sewage filters Gage and Clark[2] have already reported considerable success.

In conclusion we wish to express our thanks to Dr. Edward Bartow for his invaluable co-operation in the development of the theoretical and practical considerations involved in the work and to Mr. P. C. Jeans and Mr. J. M. Lindgren who have rendered material assistance in carrying on the analytical work.

[1] Prescott and Winslow, *Elements of Water Bacteria*, New York, 1904.

[2] *Eng. News*, 1905, 53, p. 27, and *Jour. Am. Chem. Soc.*, 1905, 27, p. 327.

THE RELATIVE IMPORTANCE OF STREPTOCOCCI AND LEUCOCYTES IN MILK.

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IN the general crusade for the public weal that has been carried on in this country for the last decade, much has been accomplished in providing for a more wholesome milk-supply in several of the great centers of population. Of especial interest in this connection are the records of the Department of Health of the City of New York[1] which show what has been accomplished in that place, and demonstrate what can be done elsewhere if apathy, ignorance, and civic wrongdoing could be overcome.

Notwithstanding advances, there yet remain problems to be solved and differences of opinion to be adjusted before it can be said that our ideas of fitting sanitary standards have been crystallized or brought into unison.

Probably two of the most important questions at present under discussion are those concerned with the presence and significance of streptococci and of "pus cells" in milk-supplies. Thus, according to the standards raised by one authority or another, a milk is adjudged good or bad. In this confusion of what should be considered a proper standard lie the difficulties and differences of opinion. To the writer it seems that the time is now ripe for a full and free discussion of the subject, and that a careful inquiry should be instituted to consider the problem in all its phases and to advise regarding the establishment of suitable standards.

The scope of this paper will be limited to a brief presentation and discussion of these topics, with criticisms and suggestions.

It is now generally agreed that it is a rare thing to be able to draw sterile milk in any moderate quantity from the udders of cows, even while using the greatest of precautions against contamination from the outside; witness the researches of Ward,[2] Reed and Ward,[3] Boekhout and de Vries,[4] von Freudenreich,[5] Conn,[6] Harrison and Cumming,[7] and many others. Such being the case, it is important

to know of what species are those bacteria which inhabit the milk ducts and teat-canals.

These may be divided into two main groups; first, those producing lactic acid fermentation, and second, those producing none. Of the former there exist two types, one giving rise during the fermentation to gas, commonly spoken of as the "*B. aerogenes* type;" the other giving rise to no gas and spoken of as the "*B. lactis acidi* type." Of the latter, i. e., the non-acid group, there exists a variety of forms that are apparently concerned in the causation of odors, flavors, etc., and which at times may even give rise to putrefactive changes. By far the most common, however, of all those bacteria are the lactic acid forms which in market milk, according to many authorities, run from 50 to 100 per cent, and in milk drawn from the udders, according to Harrison and Cumming, 95 per cent.[7]

Concerning the constitution of these acid-forming types, it is generally agreed that to the "aerogenes" type belong *B. aerogenes* (Escherich) for the most part, and in less degree *B. coli;* but all investigators are not of one mind concerning the identity of the bacteria entering into the type "*B. lactis acidi*" (Leichmann).[8] The literature, for example, contains descriptions of organisms under such names as *B. acidi lactici*, *B. lactis acidi*, *Mic. acidi lactici*, *Streptococcus acidi lactici*, *Streptococcus lacticus*, etc., all concerned in the production of lactic acid and coagulation of casein.

A careful consideration of the descriptions of these several bacteria, as given in the literature, leads one to believe that the points of difference lie largely in relatively insignificant variations induced by the non-uniformity of media, or of technique, or in questions of interpretation of results.

The question of the probable unity of the varieties described was first prominently brought forward by Kruse[9] of Bonn in 1903, who seriously doubted the bacillary nature of the lactic acid bacteria of the "*B. lactis acidi*" type, declaring the same to be streptococci closely related on the one hand to *Strept. pyogenes*, and on the other to *Mic. lanceolatus*. His paper, however, was not accompanied by any experimental data, although it must be inferred that he had conducted experiments leading him to this belief. He assigned a secondary rôle to *B. aerogenes* and *B. coli* as lactic acid formers in naturally soured milk.

In the following year there appeared from his laboratory a contribution on this subject by Hölling,[10] containing much experimental evidence of a convincing nature, wherein it is shown that streptococci are in reality the ordinary and most frequent lactic acid formers, and that *B. lactis acidi* and others of that ilk are not bacilli, but streptococci. With the exception of *B. aerogenes* and *B. coli*, this statement covers all previously described organisms recognized by various authorities as being those concerned in the primary lactic acid fermentation of fresh milk.

In ignorance of the papers of Kruse and Hölling, Heinemann,[11] working in the University of Chicago, in 1905, carried out examinations upon milk obtained from the udders of 42 cows under the best of precautions against contamination from the outside, and likewise upon samples of market milk in Chicago, with the object of ascertaining the nature of the bacteria concerned in the souring of milk, together with an inquiry into the status of "*B. lactis acidi.*" For this latter part of the work pure cultures of this bacterium were obtained from several laboratories in this country, and also a series of "starters" such as are sold to dairymen for the ripening of cream. In all these investigations he found that there was but one organism concerned, and that organism was not a bacillus but a streptococcus, and, that in milk naturally soured, forms corresponding to *B. aerogenes* and *B. coli* were numerically less frequent. In this work he confirmed the remarks of Kruse[9] and the experimental data and conclusions of Hölling.[10] Further, he succeeded in isolating from separator slime, cows' feces, and in washing from the cows' udders and teats lactic acid bacteria corresponding in all particulars with the foregoing streptococcus type.

It is not to be inferred that, previous to these investigations, observers had not recorded the occurrence of streptococci in milk. The literature, which need not here be quoted, teems with records of such. But what seems strange is the fact that none regarded his results as other than an expression of a pathological state of the cow's udder, whilst "*B. lactis acidi,*" regarded as a normal inhabitant, was passed by unnoticed as being of the same species, largely it would seem upon the ground of morphology, the bacterium occurring in pairs or short chains of slightly elongated individuals, an expression

of active binary division often seen in cultures of pathogenic streptococci.

In so far as morphological and cultural characters were concerned, Heinemann[11] found, on comparing a series of streptococci from pathological sources, milk, pure cultures of "*B. lactis acidi*," "starters," feces, etc., that there was perfect accord; in all, to be sure, there occurred from time to time variations of a parallel nature in both conditions.

Now, if we regard "*B. lactis acidi*" and *Strept. lacticus*, as Kruse and Hölling call it, to be identical (as I believe they are), in what light, then, are we to regard the presence of streptococci in milk as being an index of disease in the cow? It cannot be denied that cows suffer from inflammation of the udder at one time or another during lactation, and that these lesions are largely caused by the ordinary pyogenic cocci, less frequently by *B. coli* or *B. aerogenes*. Of the cocci it would seem that a streptococcus is the more prominent factor, judging from the researches of Steiger,[12] Bergey,[13] and others, particularly in that form of mastitis known as acute contagious mastitis.

But were we able to distinguish safely the several races of streptococci, it would then be a relatively simple matter to frame standards whereby we might be able to say that a given sample of milk was fit for consumption, whilst another was not, on the grounds that each contained harmless and hurtful cocci, respectively. In this matter we are yet without a test that will permit of assuming a dictatorial attitude. Culturally, no peculiarities of a sufficiently stable nature are apparent whereby any one race of streptococci may be sharply marked off from another. Gordon[14] has proposed the use of such fermentable substances as saccharose, inulin, raffinose, mannit, salicin, coniferin, etc. By their use he believes that he is able to say that this coccus is of salivary origin, that of fecal or pustular; in this way milk streptococci are separated from salivary races by their ability to ferment salicin. On this account Savage[15] regards this test as being of great value in identifying streptococci of bovine origin, but as there occur at times exceptions to this rule, the test, like many of a similar nature already known to us, can then be considered as a presumptive test only, if even that.

Other tests have been proposed and tried out, notably the hemo-

lytic, introduced by Schottmüller,[16] but have led to discordant results. Baumann[17] found that, with a series of pathogenic and non-pathogenic streptococci, the former were uniformly and markedly hemolytic, whereas the latter possessed no hemolytic power; amongst the non-hemolysers he classed the milk streptococci. Müller,[18] comparing the milk streptococci with those from pathological sources, noted that hemolysis occurred amongst both types, and regarded the test as one showing no differences of a sufficiently important nature. He quoted also from the literature in support of this view.

No better also are the tests in the field of agglutination. It is generally conceded that among pathogenic forms no reliable deductions as to possible identity of races can be drawn. Bergey[13] tested the method with a large number of races of milk streptococci using sera prepared from pathogenic varieties, but was unable to come to any satisfactory conclusions. Müller[18] obtained both positive and negative results among a series of pathogenic streptococci tested with pathogenic (homologous) sera, and in lesser degree among races of milk streptococci tested with the same sera. He concluded, however, that a positive result obtained with milk streptococci speaks strongly for the race being pathogenic, but a negative result leaves the question an open one. He is of the opinion that among the milk streptococci are some which are closely related to the pathogenic varieties. Baumann[17] and Savage[15] lay no stress upon agglutination as a means of differentiation, on account of its unreliability.

Tests of pathogenicity, too, are by no means a safe guide in determining the virulence of the races isolated. Beck[19] recorded that not a few of the streptococci isolated by him from the market milk of Berlin were possessed of considerable lethal power toward rabbits and guinea-pigs. Escherich, too, determined that certain races were pathogenic for white mice. But it is a well-known fact that not infrequently streptococci isolated from a variety of pathological sources in human disease produce most irregular results upon inoculation into laboratory animals, and similarly with milk streptococci we find that Reed and Ward[3], Lammeris and van Harrevelt,[20] Brüning,[21] Seiffert,[22] and Bergey[23] (1901) record the statements that the organisms isolated by them were quite avirulent to their laboratory animals, chiefly rabbits and guinea-pigs. And here we must

agree with the statement of Rullmann and Trommsdorff,[24] speaking of this phase of the subject, that such a line of work argues nothing for the pathogenicity of these streptococci toward man, and our deductions must be drawn from another line of facts.

Despite, then, our inability to distinguish racial differences among the streptococci, we cannot but feel sure that there are present in the milk from time to time, cocci which undoubtedly have a virulence all their own toward the human species, and it is the belief of those who are competent to express an opinion that these cocci thus causing human infection are those giving rise to the acute contagious variety of mastitis, or *gelber Galt* of the Germans. Rullmann and Trommsdorff, however, are of the opinion that the presence of streptococci in freshly drawn milk, especially if accompanied with leucocytes, is a sign of a chronic mastitis and the milk is to be regarded with suspicion. In this they agree with the previous statements of Bergey.

The type of disease in human beings occasioned by an infection by such milk takes on two forms, the one marked by distinct and severe gastro-intestinal disturbances combined with general depression and malaise; the other form manifesting itself in a severe faucial angina, tonsilitis, a swelling or suppuration of the submaxillary or cervical glands, occasionally cellulitis, and well-defined constitutional disturbances. Such infections usually take the form of local epidemics confined to the region supplied by the infected milk. Petruschky and Kriebel[25] are of the opinion that the streptococci are responsible for much of the mortality in that form of disease known as summer diarrhea of infants. Examples of infection supposedly caused by these streptococci are given by Holst,[26] Stokes and Wegefarth,[27] Kenwood,[28] and Savage.[29] The latter states that, in an outbreak of sore throat in Colchester, he discovered a cow in the herd of the suspected dairy affected with acute mastitis, and upon removal of the sick animal the epidemic subsided; the milk of the animal contained enormous numbers of streptococci and pus cells. Under such circumstances, then, the discovery of the cause rests, not on bacteriological grounds alone, but most largely upon a clinical examination backed up by bacteriological and microscopical findings.

To face now the question of the importance of the presence of

streptococci in milk, we must acknowledge the correctness of the view that there exists in the udders of practically all normal cows a certain species of bacterium, in numbers greatly exceeding others, and that this bacterium performs a kindly service to the dairyman and is regarded as inoffensive. Then, too, we have to accept the facts recorded by many sanitarians that there are to be observed in the milks of different countries streptococci varying in quantity from 50 to 76.6 per cent of all bacteria present. But have we to subscribe to the interpretations so often attached to the finding of these bacteria, viz., that it is evidence that the cows giving the milk are diseased and that the milk is in consequence unfit for the use of the human subject? I think not. For if we did, then we would be bound to acknowledge that for the greater part milch cows suffer more or less continuously from inflammation of the udder, which is the inference drawn from reading the experiments of Bergey,[13] and Rullmann and Trommsdorff.[24] Kaiser,[30] and Savage,[15] on the other hand, both of whom have gone into examinations of milk and found streptococci to be present in most samples, have not cared to express an opinion in this matter.

The solution of the difficulty seems to me to be furthered if we choose to accept the views of Kruse,[9] Hölling,[10] and Heinemann,[11] namely, that the normal lactic acid bacteria of the udder are not bacilli, as most investigators have thought, but streptococci, and streptococci which, under the conditions in which we find them, are, as has been shown, for the most part non-pathogenic. Then, too, as to their origin, we might indulge in some profitable speculation. Steiger[12] states that there are three routes whereby an infection of the udder may take place: (1) direct infection through the teat-canals, constituting the galactogenous infection; (2) infection through wounds of the udder or teats, on the outside, by way of the lymphatic vessels, the lymphogenous infection; (3) the hematogenous infection, occurring in the course of a general infection having a local starting-point in some distant part of the body. Whatever, then, the mode of infection and the degree of infection produced (leaving aside the question of the acute contagious mastitis), might it not be assumed that after a time the cocci gradually part with their pathogenic powers, and, undergoing some modification, give themselves over to a sapro-

phytic existence, comparable to that led by bacteria in the mouths and intestines of the human subject? Again, it may not be necessary to imagine that an infection takes place according to any of the foregoing ways. It can as readily be conceived that, by a simple fouling of the teats in a "leaky" cow by dung or other dirt, growth of already saprophytic streptococci is permitted to occur along the teat-canal into the udder; or, in a non-"leaky" animal, by a similar style of infection directly after milking, when there yet remains within the orifices of the canals a sufficiency of milk to afford the proper conditions of growth.

That the foregoing arguments for the acceptance of the view that streptococci in milk have not the significance usually attached to their presence will satisfy or cover all points of criticism, I do not pretend to assume. The stand taken is simply put forward in the endeavor to bring into greater harmony facts that appear to be unnecessarily at variance and the cause of so much doubt, as well as of doubtful or seemingly divergent interpretations. The importance of the theme is not insignificant, and if the criticism engendered by this paper will lead to a careful reinvestigation of our facts, we may arrive, it is to be hoped, at no distant day to a much clearer point of view than is at present held regarding the status of the presence of streptococci in milk.

There yet remains, however, another question closely linked up with the preceding, and one, too, that on account of much divergence of opinion requires almost as great attention at the hands of investigators, and that is the presence of the so-called "pus cells," or, more properly, leucocytes in milk.

Too much has been made of this question upon what appears to me to be rather shallow grounds; and, in addition, the somewhat unfortunate choice of the term "pus cell," instead of leucocyte, is to be regretted. As Savage[15] remarks, "That milk should not contain pus cells few will deny, but what constitutes pus in milk? All milk contains leucocytes. When does a leucocyte become a pus cell, and what distinguishes one from the other?" Such is a well-merited challenge to the propriety of the use of the word "pus," as applied to the presence of white blood cells in milk. A decided expression of opinion upon this point is urgently needed, for it must be recognized

that the presence of leucocytes in milk up to a certain point is largely a physiological circumstance; beyond that, a pathological one. What are the bounds to be set in recognizing either of these conditions? Frankly, we do not possess sufficiently reliable data upon which to dogmatize. Bergey,[13] Doane,[31] Savage, and Rullmann and Trommsdorff[24] have demonstrated that in apparently healthy cattle cell numbers may vary among different cows, and in the four mammary quadrants of the udders. To what this variation is due is hard to say. Bergey, and Rullmann and Trommsdorff find that there is a proportional relationship between leucocytes and streptococci, a high leucocyte count usually being accompanied by a correspondingly high streptococcus count; but this state of affairs was not always found to be uniformly so. Savage, on the contrary, could not find any relationship whatever. Whether, apart from any signs of inflammation, chemiotactic substances play any part in leucocytosis due to the residence of saprophytic streptococci within the udder is not known; perhaps they do.

It is interesting to trace the attempts to put this question of leucocytosis upon a practical working basis. Apparently Stokes and Wegefarth[27] in 1897 were the first to propose a plan for the estimation of leucocytes in milk, and with some modification their system is in vogue today in many laboratories in this country. Briefly it was as follows: Ten c.c. of a well-shaken sample of milk were measured out and centrifugalized in a hand centrifuge for five minutes, the supernatant fluid poured off, and a platinum loopful of the sediment spread over a slide, fixed by heat, cleared by ether, and stained with methylene blue and examined under an oil-immersion lens; the number of leucocytes were counted in 10 fields of the microscope, and if they ran over five cells per field, the milk was to be adjudged to contain pus. In 1899 Eastes[32] reported in an indefinite manner upon the presence of pus in milk. Bergey[13] in 1904, using the Stokes-Wegefarth method, decided that if the number of cells were greater than 10 per field, it was indicative of the presence of pus. Stewart[33] used the sediment from 1 c.c. of milk and set the limit of cells at 22 per field. Slack[34], using 2 c.c., spread the sediment over 4 sq.cm. of the surface of a slide, and allowed 50 cells per field of the oil-immersion lens as indicative of pus formation.

Writing in 1905 under the title, "The Doane-Buckley Method of Determining Leucocytes," Doane[31] criticizes the foregoing methods in these words: "There is but one element of scientific accuracy in the whole process. . . . the using of a definite quantity of milk"—which criticism seems to be largely justified. The later modifications differ only in the attempts to have a more or less definite area of the slide covered with sediment, which in fact adds little to the accuracy of the technique. Doane describes the technique of the Doane-Buckley method as follows: 10 c.c. of milk are centrifuged for four minutes in a graduated centrifuge tube at an approximate speed of 2,000 revolutions per minute; the cream is then lifted out with a cotton swab and centrifuged for another minute; the cream is again taken out as

before, and, without disturbing the sediment, the overlying milk is syphoned off, leaving fluid to the depth of ⅛-inch above the sediment; two drops of a saturated alcoholic solution of methylene blue are now added, mixed thoroughly with the sediment, and set aside in boiling water for two or three minutes to allow the leucocytes to take up the stain; hot water is then poured in to fill up to the 1 c.c. mark on the graduated tube; the contents are now shaken vigorously and a portion taken up in a capillary pipette and placed upon the ruled surface of a Thoma-Zeiss blood counter and covered with the cover-glass; allowing a minute for the cells to settle down, a total count of all the squares is made, or, if the cells are very many, then an average of the whole number may be obtained by the counting of say 10 or 15 squares. Calculation of the cells per c.c. of the milk is then made. The fluid over the total squares represents 0.1 c.mm., or 0.0001 c.c. Then say that there were 50 cells counted in the whole ruled part of the slide, which is equivalent to 500,000 cells, but as this number came from 10 c.c. of milk, 1 c.c. contains 50,000 leucocytes. This method certainly is free of any criticism upon the grounds of lack of scientific accuracy, as it is plainly that method used by clinicians in the enumeration of the cells of the blood with slight modifications to suit the conditions.

Ward[35] upholds the accuracy of the Doane-Buckley method in an article published this year, stating that it gives much more satisfactory results than that of Stewart, the count running from 4 to 40 times higher.

Quite independently of Doane and Buckley, Savage[15] this year has practically worked out the same method, varying the details in some degree. He, too, felt the burden of the inaccuracy of the Stokes-Wegefarth plan and so set about to develop one more suitable and more scientifically precise. As carried out by Savage, the process consists in taking 1 c.c. of the sample and putting it into a 20 c.c. graduated centrifuge tube, filling up to the 20 c.c. mark with Toisson's solution; after mixing well, the sample is centrifuged for 10 minutes, the cream is broken up thoroughly with a glass rod, and again whirled for 10 minutes. All the fluid is now removed but the last 1 c.c., which is stirred up well and the required amount is placed upon a Thoma-Zeiss counter and counted, the calculation of the cells being done by means of a special formula and rendered in cubic millimeters, thus making the counting part of the method apparently unnecessarily difficult, although its accuracy cannot be called into question.

Another system developed in Germany by Trommsdorff[36] and published this year differs in all respects from any of the foregoing, and although it is more accurate than the Stokes-Wegefarth method, it is yet only capable of furnishing qualitative results, and not quantitative as should be the case, such as is afforded by Doane and Buckley's and by Savage's methods. In this method 5 c.c. of milk are taken and put in specially devised tubes, which are drawn out to capillary points and there carefully etched into divisions of 0.001 c.c., running from 0.001 to 0.02 c.c. in volume. Upon being centrifuged for a few minutes, the amount of sediment is read off and the amount calculated in volumes per 1,000 or 10,000 as desired. It can readily be seen that these results, qualitative as they are, may be invalidated by the presence of foreign matter blocking up the capillary tube, although the inventor claims that under his care the instrument is quite free from this objection. Its usefulness may possibly be further impaired by the accumulation of other material in the milk than pus, e.g., fibrin and mucin.

Of these several methods devised for the recognition of pus in milk only three merit any consideration, namely, those of Doane and Buckley, of Savage, and of Trommsdorff. In point of accuracy and true analysis, the first two methods and the last stand in parallel contrast with each other, exactly as do the two methods for the estimation of leucocytes in the blood, i. e., by actual count and by the hematokrit; and of them, the one freer from error and more desirable is that by actual count.

To go into the results obtained by the authors would be to lengthen this paper very considerably. Suffice it to say that each finds much variation in the leucocyte count among individual cows, between the four mammary quadrants of each cow, and between individual cows' milk and that of the whole herd. As regards the question of standards, Rullmann and Trommsdorff consider that any sample yielding over 1 volume per mille is evidence enough that there is pus in the milk. To quote Savage,[15] "I cannot differentiate between a leucocyte and a pus cell, and I am not prepared at this stage to lay down an arbitrary standard as to what number of leucocytes per cb.mm. is to be designated *pus* in the milk." He also affirms that there is no relation between leucocytes and streptococci, so far as his figures go. Doane,[31] on the other hand, is more specific and states that a milk containing 500,000 cells per c.c., together with the presence of fibrin, is to be regarded as suspicious, while a content of 1,000,000 per c.c. associated with fibrin is conclusive of the presence of pus, i. e., evidence of mastitis.

This, then, closes the presentation of a subject that very evidently is at present in a rather chaotic state, but one that invites our further careful scrutiny, deliberation, and judgment, before it can be said that the milk problem is a settled one.

The following conclusions are offered:

1. It seems imperative to secure an early adjustment of the divergence of views of the taxonomic status of the so-called *B. lactis acidi.*

2. The statements of Kruse, Hölling, and Heinemann cast considerable doubt upon the value heretofore entertained regarding the significance of streptococci in milk.

3. It is not excluded by the evidence that pathogenic streptococci are to be found at times in milk; in fact, recorded observations make

this certain, the contamination arising from clinically recognizable cases of mastitis in the herds.

4. We are not as yet in possession of any reliable method for distinguishing a non-pathogenic from a pathogenic streptococcus.

5. The sanitary significance of the so-called "pus cell" has been greatly overrated. More scientific attention should be given to the study of the phenomena of lactic leucocytosis, together with a more accurate method of enumeration, such as that of Doane and Buckley, or of Savage.

6. Particularly, should more attention be given to veterinary inspection of the cows' udders, with less absolute dependence upon laboratory examination of milk for signs of infectious processes.

7. The time seems ripe for throwing open the whole question for discussion, and the framing of new rules to cover points raised and accepted.

REFERENCES.

1. *Ann. Rep. Dept. of Health, City of New York for 1902*, p. 317.
2. WARD, A. R. *Bull. No. 178, Cornell Univ. Agri. Exper. Sta.*, January 1900.
3. REED, R. C., and WARD, A. R. *Amer. Med.*, 1903, 5, p. 256.
4. BOEKHOUT, F. W. J., and DE VRIES, J. J. O. *Centralbl. f. Bakt.*, Abt. 2, 1901, 7, p. 817.
5. VON FREUDENREICH, E. *Die Bakt. in der Milchwirtschaft*, 3d. ed., 1906; Jena, G. Fischer.
6. CONN, H. W. *15th Ann. Rept. Storrs Agri. Exper. Sta.*, 1903, p. 92.
7. HARRISON, F. C., and CUMMING, M. *Jour. Applied Microsc.*, 1902, 5, p. 2029.
8. LEICHMANN, G. *Centralbl. f. Bakt.*, Abt. 2, 1896, 2, p. 777.
9. KRUSE, W. *Ibid.*, Abt. 1, 1903, 34, p. 737.
10. HÖLLING, A. Inaug.—Dissert., Univ. of Bonn, 1904.
11. HEINEMANN, P. G. *Jour. Infect. Dis.*, 1906, 3, p. 173.
12. STEIGER, P. *Centralbl. f. Bakt.*, Abt. 1, 1903–4, 35, pp. 326, 467, 574.
13. BERGEY, H. D. *Bull. No. 125, Depart. Agri., Commwlth. of. Pennsylv.*, 1904.
14. GORDON, M. H. *Lancet*, 1905, 2, p. 1400.
15. SAVAGE, W. G. *Jour. Hyg.*, 1906, 6, p. 123.
16. SCHOTTMÜLLER, H. *Münch. med. Wchnschr.* 1903, 50, p. 909.
17. BAUMANN, E. *Ibid.*, 1906, 53, p. 1193.
18. MÜLLER, P. T. *Archiv f. Hyg.*, 1906, 56, p. 90.
19. BECK, M. *Deutsch. Vierteljahrsschrift f. offent. Gesundhtspflg.*, 1900, 32, p. 430.
20. LAMMERIS, and VAN HARREVELT. Ref. in *Centralbl f. Bakt.*, 1901, 30, p. 83.
21. BRÜNING. Quoted by Kaiser (*vide infra*).
22. SEIFFERT. Quoted by Kaiser (*vide infra*).
23. BERGEY, H. D. *Amer. Med.*, 1901, 1, p. 122.
24. RULLMANN, W., and TROMMSDORFF, R. *Archiv f. Hyg.*, 1906, 59, p. 224.

25. PETRUSCHKY, J., and KRIEBEL, M. *Die Ursachen der Sommersterblichkeit der Säuglinge u. die Möglichkeit ihrer Verhütung;* Leipzig, Leineweber, 1904.
26. HOLST, A. *Festskrift til Prof. Heiberg,* 1895; ref. in Baumgarten's *Jahresbrcht.,* 1895, 11, p. 52.
27. STOKES, W. R., and WEGEFARTH, A. *Jour. of State Med.,* 1897, 5, p. 439; *Med. News,* 1897, 71, p. 45.
28. KENWOOD, H. *Brit. Med. Jour.,* 1904, 1, p. 602.
29. SAVAGE, W. G. *Ibid.,* 1905, 1, p. 1165.
30. KAISER, M. *Archiv f. Hyg.,* 1906, 56, p. 51.
31. DOANE, C. F. *Bull. No. 102, Maryland Agri. Exper. Sta.,* 1905.
32. EASTES, G. L. *Brit. Med. Jour.,* 1899, 2, p. 1341.
33. STEWART, A. H. *Amer. Med.,* 1905, 9, p. 486.
34. SLACK, F. H. *Jour. Infect. Dis.,* 1906, Supplm. 2, p. 214.
35. WARD, A. R. *Science,* 1906, n. s. 23, p. 211.
36. TROMMSDORFF, R. *Münch. med. Wchnschr.,* 1906, 53, p. 541.

LEUCOCYTE STANDARDS AND THE LEUCOCYTE CONTENT OF MILKS FROM APPARENTLY HEALTHY COWS.

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OF late years an increasing amount of attention has been given to the determination of the sanitary properties of milk supplies. The old standards, based largely upon the sense of smell and taste, are no longer regarded as the ultimate basis for a proper judgment as to the quality of milk. Various new methods have been introduced for the purpose of controlling the character of these supplies. Those so far proposed, however, have been developed for the detection of two different sets of conditions:

(1) The determination of the presence of organisms that have found their way into milk mainly through careless methods of handling.

(2) The detection of abnormal conditions in milk arising from its condition in the udder.

The first type of standards embraces those relating to the degree of cleanliness with which the milk is handled; the second concerns the healthfulness of the milk as it comes from the cow.

Among the more important standards that have been proposed may be mentioned, under Group (1), the following:

(*a*) The determination of the total germ content by cultural methods, a method which has been adopted by a considerable number of cities.

(*b*) The determination of the total germ content by direct microscopic examination, as proposed by Dr. Slack of the Boston Board of Health.

(*c*) The use of the Wisconsin curd test, by means of which is recognized the presence of gas-producing and taint-forming bacteria, organisms associated with careless methods of milk-handling.

Under Group (2) are included such test methods as reveal—

(*a*) The presence of special types of organisms, such as streptococci, that are presumed to be associated with the production of certain udder diseases.

(*b*) The supposed presence of pus, by the determination of pus cells, or the detection of associated elements, such as fibrin.

Each of these methods that have been suggested has elements of value, but in the present state of development, it is a question whether any absolute standard can be proposed that is a satisfactory basis for milk inspection. The actual detection of disease-producing organisms in milk is exceedingly difficult under most conditions. Fortunately there are not many diseases which occur in the cow that affect man. Tuberculosis is by far the most important of this class.

On the other hand, cattle not infrequently suffer from udder troubles—gargets of various types, in which inflammatory processes of varying degrees of severity may occur. In some cases these may be so slight as apparently not to affect the nature of the milk. Often, however, the milk becomes viscous, sometimes clotted or stringy, and may even be of a bloody nature. Occasionally such troubles as these develop into a gangrenous stage, in which pus is actually present in such quantities as to be easily recognizable. Milks of this character should, of course, be excluded from food supplies. In these cases, where a physical examination reveals an abnormal condition, a microscopic study naturally presents a far different picture from that which obtains in apparently normal milk. One of the prominent microscopic features in the case of milk that is abnormal at time of withdrawal is the presence of an unusually large number of certain blood elements, known as the white blood corpuscles, or leucocytes. In case pus is at all observable, these cellular elements are present in enormous numbers. Generally they can be detected, even where no physical signs of pus are apparent. The fact that milks known to be associated with abnormal conditions in the cow often contain these elements in such large numbers has led to the suggestion that all milks rich in leucocytes should be regarded with more or less suspicion.

Histologically, it is not an easy matter to differentiate between pus cells and the normal leucocytes, or white blood corpuscles. These latter cellular elements are, of course, normal constituents of blood, and are also found in variable quantities in milk supplies. It is quite probable that some of these cellular elements found in milk are derived from the gland tissues and are not leucocytes, although, upon staining, the majority of the cells are polymorpho-

nuclear. Thus far, emphasis has been placed on the quantitative rather than the qualitative relation of these cells in milk. Whatever may be their nature, they are at present considered as associated with pus, if they are found abundantly in the milk.

The presence of a large number of leucocytes, either in blood or milk, is regarded as indicating an irritation of an inflammatory character, but the important question is, what constitutes an abnormal number? How many leucocytes can be present in milk before it should be considered that suppuration has taken place? The standards, so far chosen, have been more or less arbitrarily selected on what appears as rather inadequate data, and, from a comparison of results, it is apparent that much more comparative work needs to be made. Even the technical methods by which the leucocytes are determined do not show harmonious and constant results, and the interpretations placed upon the data obtained vary still more.

The formulation of these standards for milk inspection has already led to much dissatisfaction and complaint on the part of the dairy interests. In a number of cases, even certified supplies which have been handled with great care have been rejected on account of the high leucocyte content found in them, although the closest veterinary inspection failed to reveal the presence of any udder abnormality. We quote from a recent correspondent in Tennessee:

> The city chemist of Memphis is taking steps to condemn 35 to 75 per cent of the dairy cows of this county for alleged discovery of pus in the milk. He has condemned 16 out of 35 in our herd, and in the case of some dairymen, 90 per cent of their herds. The cows are in good condition and the veterinarian from the University of Tennessee has examined my herd, and pronounced their general condition good, and their udders perfect. There is absolutely no sign of inflammation.

In a letter from Doctor S. McC. Hamill of Philadelphia, who is the secretary of the Milk Commission of the Pediatric Society of that city, he says:

> Some of the best herds have been publicly condemned and brought into disrepute. It has not been shown by anyone that milk high in leucocytes has any injurious effect, nor has it been shown that their presence meant *per se* inflammatory changes in the udder.

As secretary of this commission, Doctor Hamill enters a vigorous protest against the attitude of the city board of health, which is enforcing a standard whereby milk supplies are condemned in which are found 100,000 or more leucocytes per c.c.

METHODS OF EXAMINATION.

Before discussing the standards proposed it is pertinent to study the methods of examination which so far have been devised.

Naturally the number of blood elements can best be determined by the use of the centrifuge. In the main, two methods have been suggested by which the leucocytes can be quantitatively determined; viz.:

(*a*) *Smeared-sediment method.*—This method, devised by Stokes[1] of the Baltimore Board of Health, and later somewhat modified by Stewart of the Philadelphia Bureau of Health, consists in centrifugalizing a definite quantity of milk in rubber-stoppered tubes, pouring off the cream and milk, rubbing up the collected sediment with water, and smearing this emulsion on a glass slide of definite dimensions. The preparation after staining is examined by a one-twelfth inch oil immersion lens, and the number of leucocytes per field noted.

(*b*) *Direct quantitative method.*—This method was suggested by Doane[2] and is carried out in essentially the same way as the generally accepted quantitative method of determining the blood cells. Ten c.c. of milk are centrifugalized in sedimentation tubes. The cream is then removed by means of a cotton swab and the material treated in the same manner a second time, thus wholly eliminating the fat.

The supernatant milk is then siphoned off. Water is added to the collected sediment, bringing the same to the one c.c. mark, and the whole thoroughly emulsified. Quantitative determinations are made from this material in a ruled blood counter (Thoma-Zeiss pattern).

Savage[3] has recently studied the relation of streptococci and leucocytes in milk and has employed a method quite similar to that of Doane. His technique was evidently developed independently, as he refers only to Stokes' method in discussing the subject.

Stokes' method has been adopted by most investigators who have studied this question. Bergey at Philadelphia has carried on quite an extensive series of examinations by this method, except that he substituted a cover-slip for the glass slide of given dimensions. The health boards of Philadelphia and Boston, the two cities that have been the most active in the examination of milks as to leucocytes, have both adopted this method.

The objections to this technical procedure are quite apparent. It is impossible to remove wholly the sediment from the rubber corks, and it is quite out of the question to distribute this material uniformly enough over the glass surface so that duplicate specimens can be secured which will give consistent results. This distribution can be more effectively obtained by emulsifying the sediment with a small quantity of water. Moreover, this treatment requires staining the cells, and if the leucocytes are not spread in an exceedingly thin layer, it is impossible to count them accurately. In our experiments there appears to be but little question as to the comparative merits of the two types of methods that have been proposed.

[1] "The Microscopic Examination of Milk," *Jour. of State Med.*, 1897, 5, p. 439.

[2] *Bull. 102, Maryland Agri. Expt. Sta.*, 1905 (often referred to in the literature as the Doane-Buckley method).

[3] *Jour. Hyg.*, 1906, 6, p. 123.

COMPARISON OF METHODS OF EXAMINATION.

In order to determine their relative accuracy, a considerable number of duplicate examinations were made upon the same sample of milk by the Doane-Buckley and the Stokes-Stewart methods. These data are incorporated in Table 1, from which it appears that

TABLE 1.

COMPARATIVE EXAMINATION OF LEUCOCYTES PER C.C. IN MILK, AS DETERMINED BY THE TWO METHODS PROPOSED.

	VOLUMETRIC (DOANE-BUCKLEY) METHOD				SMEARED-SEDIMENT (STEWART) METHOD			
Sample	First Examination	Second Examination	Average	Percentage Variation	First Examination	Second Examination	Average	Percentage Variation
1.....	590,625	700,000	645,310	18.0	403,200	238,500	320,850	69.0
2.....	325,000	288,750	306,875	12.0	16,200	113,400	64,800	587.0
3.....	1,012,500	975,000	993,750	3.7	99,100	154,000	126,560	55.4
4.....	11,250	12,500	11,875	11.1	4,500	16,200	10,360	260.
5.....	701,250	687,500	694,375	2.0	82,870	146,830	114,850	77.1
6.....	146,250	155,000	150,625	5.6	136,020	120,700	128,360	12.6
7.....	20,000	20,000	20,000	0.0	19,810	18,900	19,370	4.7
8.....	193,750	187,500	190,625	3.3	153,000	111,700	132,800	37.7
9.....	881,250	901,250	891,250	2.2	366,300	517,500	441,900	41.2
10.....	50,000	50,000	50,000	0.0	0	9,000	.....	....
11.....	42,500	51,250	46,875	20.0	63,000	76,560	69,810	21.4
12.....	413,750	422,500	418,125	2.0	135,210	150,430	142,820	11.2
13.....	10,000	10,000	10,000	0.0	2,250	13,510	8,880	500.0
14.....	438,750	425,000	431,875	3.2	193,670	145,930	169,800	32.5
15.....	125,000	132,500	128,750	6.0	0	17,115	.....	.....
16.....	4,680	3,900	4,290	10.0	1,350	0	.....	.....
17.....	87,500	90,000	88,750	3.0	40,530	9,000	24,760	350.4
18.....	543,750	537,500	540,625	1.1	284,650	152,235	218,440	87.0
19.....	443,750	443,750	443,750	0.0	275,640	276,545	276,095	0.3
20.....	222,500	231,250	226,875	4.3	126,110	159,440	142,775	26.4
21.....	245,000	247,500	246,250	1.0	174,750	202,680	188,720	16.0
22.....	1,076,250	1,057,500	1,066,875	1.7	439,590	545,885	492,740	24.2
23.....	142,500	125,000	133,750	14.0	71,180	87,375	79,280	22.7
				Average, 5.6%				Average, 112%

the results obtained by the volumetric method (Doane-Buckley) are very much more harmonious than those secured by the smeared-sediment method. The average percentage variation in duplicate examinations in the smeared-sediment method is very great (112 per cent), while that of the other method rarely ever exceeds more than 10 to 20 per cent, and in 23 tests averaged less than 6 per cent.

It is noteworthy in the above that, quantitatively, higher results were almost uniformly obtained by the Doane-Buckley method than by the Stokes-Stewart method. This fact should be taken into consideration in the application of any standard.

Ward has also recently reported a comparison of these two methods,[1]

[1] *Rep. California State Board of Health, 1905–1906*, p. 142.

in which similar results were obtained. From these data it appears, without question, that the volumetric method is very much more accurate for the determination of the actual conditions in milk; and not only in scientific studies on this question, but also in the practical application of any standard in public health work this should be given preference over the smeared-sediment method.

TECHNIQUE USED IN WORK HERE REPORTED.

The method which we have followed in the work to be reported is essentially the Doane-Buckley method with some slight modifications. A turbine Babcock tester was employed to centrifugalize 10 c.c. of milk in ordinary sedimentation tubes twenty minutes at 1,200 revolutions per minute. We have not followed the practice of remixing the cream and again centrifugalizing, as our experience showed so few leucocytes in the cream as to be practically negligible. Savage[1] found that the number of leucocytes in the supernatant fluid did not exceed 12 per cent of the total count.

The cream and the supernatant milk were removed, with the exception of the last half cubic centimeter, by aspirating with an exhaust pump, and wiping the walls of the tube with a cotton swab. The thoroughly mixed residue was then placed in the blood counter and examined unstained. While most observers have usually stained this material prior to examination, we have found no difficulty in working with unstained material. The cellular elements are easily differentiated, as they settle quickly to the bottom of the cell. The fat, which is a disturbing factor in such microscopic observations, is practically all removed in this process; the very few small globules which remain rise to the top of the liquid in the counting cell and do not interfere with microscopic observations.

The count is made with a No. 1 eye-piece, and a $\frac{1}{6}$-inch objective. Where the number of leucocytes exceeded 12 or 15 per microscopic field, six sets of 16 each of the smallest ruled squares of the blood counter, or approximately one-fourth of the entire area of the ruled scale, were counted. Where it was less than this, 10 to 16 such sets were counted. The average was then obtained, which, when multiplied by the factor 12,500 (the ratio between the counted volume and one c.c.), gave the total number of leucocytes per c.c.

FORMULATION OF STANDARDS AS TO PUS CONTENT.

Various numerical standards have been suggested from time to time as indicating the presence of pus in milk. A comparison of these shows that considerable variation exists, not only in the standards themselves, but in the interpretation of results.

Stokes[2] reached the conclusion that pus was present when more than ten[3] cells were found in a $\frac{1}{18}$-inch field. Bergey[4] adopted this same standard (10 per field)

[1] *Loc. cit.*, p. 129.

[2] *Rep. Health Department, Baltimore, 1899;* also *Med. News*, 1897, 71, p. 45.

[3] Since then, according to Doane (*Bull. 102, Maryland Agri. Expt. Sta.*, p. 214), Stokes has suggested the advisability of increasing this limit to 25 leucocytes for the same field.

[4] *Univ. of Pa. Med. Bull.*, July–Aug., 1904, p. 8.

in 1900,[1] and his later studies have confirmed his opinion as to the correctness of this standard.

Stewart[2] of Philadelphia has increased his standard considerably above the limits selected by Bergey. He recommends spreading the sediment over one sq. cm. and advises condemnation of the sample when the microscopic field (with $\frac{1}{12}$-inch objective and No. 3 eye-piece) contains more than 23 leucocytes per field. As it requires practically 4,400 fields to cover the one sq. cm. of the smeared preparation, this would be equivalent to 100,000 leucocytes per c.c. This standard has been quite widely accepted and is now in force in a number of cities.

Doane, using his volumetric method, suggested a materially higher standard as he found quite frequently that the milk of apparently healthy animals exceeded the 100,000 limit. He suggested 500,000, and possibly 1,000,000 leucocytes per c.c., as a preferable limit.

As pointed out above, and also by Ward, the results obtained by the Doane-Buckley method show on the same sample of milk a very much higher leucocyte content than those obtained by the smeared-sediment method. It is, therefore, very necessary that a uniform method of examination be selected before adopting any standard for comparison.

RESULTS OBTAINED IN PRACTICAL WORK ON MILK EXAMINATION.

For the last two years certain milk supplies received in Philadelphia and Boston, have been examined as to numerical leucocyte content, the presence of streptococci, and total bacterial content.

Stewart has investigated six of the best milk supplies furnished in Philadelphia—supplies that were certified by the Milk Commission of the Pediatric Society of that city, with the following results:

TALBE 2.

RESULTS OF EXAMINATION OF CERTIFIED MILK SUPPLIES OF PHILADELPHIA BY LEUCOCYTE STANDARDS (STEWART).

Herd Number	Total Number of Samples Examined	Number of Samples containing Pus in Excess of Legal Standard (100,000 per c.c.)	Percentage of Samples Condemned on Pus Standards
1.....	54	7	13
2.....	16	4	25
3.....	54	2	4
4.....	54	4	8
5.....	52	4	8
6.....	52	0	0

Slack (*Thirty-fourth Health Report*, Health Department, Boston, 1905) gives the results of an inspection of the regular supplies furnished Boston, in which 5,559 samples were tested, and 279 (or 5 per cent) of them were condemned as containing

[1] Reference to Bergey's original paper, published in the *Report of Secretary of Agriculture of Pennsylvania, 1900*, p. 123, shows that he proposed a limit of five leucocytes per $\frac{1}{12}$-inch field, and any excess of this number he believed to be associated with a pathological condition of the udder, especially if the leucocytes showed a tendency to be bunched together.

[2] *Amer. Med.*, 1905, 9, p. 486.

pus in such quantities as to be considered as infected. Presumably this condemnation was made on the basis of an excess of 100,000 leucocytes per c.c., although specific information as to this point is not presented. In this case, the method of examination was the slight modification of that used by Stokes and Stewart.

Savage, using the direct quantitative method of enumeration, examined the milk of 11 herds, or a total of 40 cows, in which in no case was there any physical symptom of udder infection. The range in leucocyte content found by him was 35,000 to 4,380,000 per c.c. Thirty-three of the 40 cows had 100,000 leucocytes per c.c., or over. An examination by him of the milk supplies taken from 17 different herds showed a range from 21,000 to 1,980,000 leucocytes per c.c. All but three herds showed a leucocyte content in excess of the 100,000 limit.

Doane has made a careful study by quantitative methods of the Station herd at the Maryland Experiment Station. Twenty-five animals were examined three times during a period of about three months. The first examination showed an excess of 100,000 leucocytes per c.c. in 14 out of 23 cases; the second examination, in 17 out of 22; and the third (two months later), in 10 out of 16 cases.

Another well-kept dairy herd near Baltimore was also examined by him and, in a single test made on 102 cows, the leucocyte content ranged from 2,000 to 4,600,000 per c.c., with an average of over 240,000. Forty-six animals in this herd showed an excess of 100,000.

It would appear from these figures that the milk of healthy animals often contains a much larger number of leucocytes than has heretofore been considered as permissible in a wholesome milk supply, but it is highly desirable that further data be accumulated, especially on individual cows whose physical condition could be more closely controlled.

RESULTS ON UNIVERSITY HERD.

No study has yet been reported on milks taken from individual animals where the examination has extended throughout a considerable consecutive period of time. Data of this sort would seem to be necessary in order to secure the normal individual range in leucocytes. The examinations which are here reported were made on samples taken from the University herd, which is composed of high-grade and pure-bred animals of representative dairy breeds. Daily examinations from the mixed-pail milk for a period of about one month were made during the summer (June and July), and then on the same animals for about two months in the fall (October and November).

This herd has been given a much closer degree of supervision than is ordinarily the case. The cows were carefully handled as to feeding, and also subjected to frequent veterinary inspection. The herd, with but a single exception, was in apparently good condition. This cow (No. 3) had chronic mammitis in one quarter.

LEUCOCYTE CONTENT OF MILK OF INDIVIDUAL

No. of Animal	Condition of Animal	June, 1906						July, 1906					
		24	25	26	27	28	29	2	5	12	13	15	16
2	Normal	37	128	....	....	....	131	....	64	31	....	29	31
8	"	....	....	5	....	....	16	....	22	1	....	....	10
9	"	....	....	1,097	....	....	306	297	....	260	382	....	501
10	"	....	39	....	....	....	....	60	....	4	....	26	22
11	"	....	115	....	....	....	....	162	....	139	....	120	54
12	"	....	....	66	....	....	....	37	....	35	....	12	12
14	"	272	....	208	....	137	....	204	....	439	372	....	456
15	"	....	....	159	....	224	....	376	....	141	297	....	306
16	"	103	61	....	47	....	....	....	55	200	67	....	44
19	"	896	....	1,026	....	35	....	1,707	....	314	1,100	....	397
20	"	....	....	122	....	....	45	87	....	95	156	....	187
22	"	....	82	....	....	....	....	56	....	19	76	....	79
24	"	....	181	....	....	186	....	92	....	214	125	....	297
26	"	....	4	....	23	82	....	....	....	....	....	7	12
27	"	58	101	....	80	59	....	81	....	350	....	164	329
28	"	....	....	....	....	....	....	....	....	....	....	....	....
29	"	....	....	....	....	....	....	....	....	....	....	....	....
30	"	....	....	....	....	....	....	....	....	....	....	....	....
1	Slight induration	11	....	115	124	....	56	....	356	162	....	226	162
3	Bad; acute mammitis	....	....	36	....	....	16	....	32	32	....	51	25
4	Udder coarse and hard	....	405	....	21	....	564	....	669	....	....	229	501
5	Slight induration	932	....	205	....	222	....	....	645	320	694	....	....
6	Indigestion	304	544	....	....	....	....	214	....	275	75	....	194
7	Slight induration	....	....	60	....	77	....	63	....	79	66	....	81
13	" "	....	47	....	39	....	32	41	20	10	....	45	107
17	" "	....	....	279	1,367	....	707	....	1,281	1,482	....	325	....
18	" "	110	427	....	227	134	....	....	312	....	464	....	357
21	" "	....	468	....	....	....	....	326	....	185	....	331	1,581
23	" "	....	....	75	4,132	....	600	....	657	589	....	1,085	804
25	" "	1,068	....	....	133	....	....	....	296	451	616	....	81

No. of Animals	Condition of Animal	October,										
		9	10	11	15	16	17	18	19	20	21	22
2	Normal	....	....	156	92	....	172	135	86	147	75	32
8	"	11	9	9	21	5	....	....	....	....	....	....
9	"	941	472	576	....	1 966	712	1.037	400	387	126	864
10	"	45	22	166	44	69	339	150	81	120	90	64
11	"	20	61	47	20	57	55	35	101	59	99	50
12	"	....	....	....	70	26	50	75	145	46	87	132
14	"	345	....	....	....	....	339	892	304	262	362	597
15	"	....	....	6	19	....	....	....	....	....	....	....
16	"	....	....	....	....	....	....	....	....	....	....	....
19	"	381	397	275	230	204	347	557	370	581	264	185
20	"	....	139	254	69	139	212	401	175	91	166	11
22	"	....	....	....	....	....	....	....	....	....	....	....
24	"	147	119	187	54	181	162	232	179	135	75	52
26	"	30	36	112	30	47	24	30	19	21	21	27
27	"	350	2.191	1.416	1,087	2,014	....	....	....	....	....	....
28	"	....	....	....	....	....	30	39	30	42	201	34
29	"	....	....	....	....	....	24	47	57	32	12	15
30	"	....	....	....	....	....	12	25	14	24	41	41
1	"	....	....	156	92	....	170	189	89	175	99	182
3	Slight induration	....	489	912	3.475	3.465	3,625	1,097	1.501	1.350	1,044	1,041
4	Bad; acute mammitis	237	304	3.945	4.952	295	....	....	....	....	....	....
5	Udder coarse and hard	362	312	122	407	664	412	785	689	1,781	581	245
6	Slight induration	91	....	225	179	220	....	....	....	....	....	....
7	Indigestion	11	57	20	....	17	7	231	41	42	82	30
13	Slight induration	107	....	62	294	172	109	66	41	35	501	1,157
17	" "	1,420	1,676	1,756	4 232	514	1,200	1.487	591	872	595	1,166
18	" "	....	1,101	1.801	160	182	89	122	87	194	160	107
21	" "	....	....	147	56	145	....	....	....	....	....	....
23	" "	62	414	172	1.595	704	....	....	....	....	....	....
25	" "	9	....	144	50	50	104	84	44	132	39	62

rs in University Herd (000 Omitted).

		September, 1906					October, 1906						Remarks
	19	24	25	26	27	28	1	2	3	4	5	8	
5	51	....	144	....	60	99	139	135	....	200	194	....	In heat July 15, '06
5	64	39	16	26	19	12	10	....	....	9	....	46	
6	320	426	322	562	594	270	197	....	....	....	....	444	Calved June 23, '06
6	232	204	74	102	212	144	87	....	....	....	....	72	Calved September 14, '05
7	131	160	64	76	87	90	132	....	....	....	....	57	Calved May 3, '06
4	56	64	72	216	15	27	11	....	....	35	106	....	
0	....	550	629	644	531	....	175	....	....	201	189	....	Calved April 5, '06
9	254	304	341	137	132	....	....	....	....	....	....	....	
2	201	41	....	126	65	216	....	....	56	117	57	....	
1	866	....	....	....	....	....	....	....	....	551	595	235	Gargety for a time in 1905 Calved October 1, '06
1	129	401	85	139	166	208	65	50	....	110	141	151	
6	41	....	....	....	....	....	....	....	....	....	....	....	
9	141	64	85	82	60	72	....	141	....	....	....	87	Milk fever June 12, '06
6	....	35	31	8	25	29	21	14	....	....	....	42	
4	157	731	419	381	491	714	....	....	1,594	1,056	1,000	....	
.	....	....	....	....	....	....	....	....	....	....	....	....	Calved August 21, '06
.	....	....	....	....	....	....	....	....	....	....	....	....	Calved July 7, '06
.	....	....	....	....	....	....	....	....	....	....	....	....	Calved September 18, '06
5	539	....	144	....	60	99	139	135	....	200	194	....	
7	56	107	206	172	125	145	2,267	....	....	1,287	1,104	669	
.	329	435	722	994	212	1,639	1,420	....	....	....	....	607	Aborted March 10, '06
5	257	682	207	282	316	797	166	....	....	....	....	275	Calved May 2, '06
7	191	210	187	169	100	407	....	157	....	160	....	....	
6	41	66	51	....	72	66	11	229	....	....	....	10	Calved March 17, '06
2	90	541	831	350	244	245	36	102	49	630	1,191	320	
5	1,162	854	326	566	395	972	150	410	....	....	....	1,266	Calved April 26, '06
5	417	....	....	....	....	....	....	....	....	....	....	....	Calved October 3, '06. Had milk-fever soon after
0	295	106	175	82	74	189	....	....	....	....	....	....	
1	660	379	197	94	144	364	100	....	....	441	154	....	
9	254	79	139	....	56	102	32	67	30	....	....	87	Calved September 18, '06

6										November, 1906			Remarks
13	24	25	26	27	28	29	30	31A.M	31P.M.	1	2	3	
121	59	77	212	157	76	222	243	229	162	210	170	404	
...	....	....	....	....	....	....	....	....	....	....	....	....	
307	241	344	560	1,060	525	262	750	531	310	791	491	564	
169	67	70	65	36	66	145	69	91	157	166	54	162	
111	100	137	102	55	137	77	41	71	86	79	77	107	
90	89	65	182	150	117	82	172	124	157	105	117	172	
301	169	419	222	276	341	619	229	291	204	300	194	164	
...	....	....	....	....	....	....	....	....	....	....	....	....	
...	....	....	....	....	....	....	....	....	....	....	....	....	
564	1,066	347	610	366	294	1,214	581	481	335	981	512	614	
137	131	216	130	297	231	132	169	412	97	162	176	231	
...	....	....	....	....	....	....	....	....	....	....	....	....	
46	169	114	136	92	99	137	91	125	61	59	92	115	
12	12	22	42	24	12	29	41	26	41	46	30	21	In heat October 12 and October 30, '06
...	....	....	....	....	....	....	....	....	....	....	....	....	
36	74	44	110	35	17	61	76	54	44	132	46	36	
15	16	19	15	19	12	21	19	73	20	49	27	37	
12	46	17	10	26	16	30	11	15	3	19	26	20	
79	269	175	151	187	210	167	250	194	150	125	142	162	
...	....	....	....	....	....	....	....	....	....	....	....	....	
...	....	....	....	....	....	....	....	....	....	....	....	....	
494	432	279	389	1,150	354	395	354	237	351	281	390	976	
...	....	....	....	....	....	....	....	....	....	....	....	....	
17	37	60	109	39	26	137	54	62	65	106	45	79	
970	654	232	275	49	52	49	....	51	30	46	25	55	
585	3,650	841	1,329	1,281	1,266	2,451	3,627	822	510	307	494	1,225	
94	156	67	75	34	32	115	110	81	191	170	87	120	
...	....	....	....	....	....	....	....	....	....	....	....	....	
...	....	....	....	....	....	....	....	....	....	....	....	....	Gargety during October, '06
46	62	65	60	51	31	172	135	185	115	350	110	125	

In order, however, to ascertain with especial care the exact condition of each animal, as to the quality and condition of her udder, the entire herd was subjected to a most rigid examination by Dr. Alexander, the Station veterinarian, and Professor Humphrey, of the Animal Husbandry Department. In this examination every abnormality, however slight, was taken into consideration. We have therefore divided the results reported below on the basis of this physical examination, including in one group all those animals which were wholly free from any udder blemish whatsoever, while in the second group are included those in which some manifestation of present or past udder trouble was observable. In this latter group, the induration often was very slight, but the previous history of the animal usually indicated that such animals had had some form of udder disturbance at some earlier date. At the time of the present examination the milk of these cows showed no abnormal condition, except in the single case mentioned. This history is especially interesting, as it may throw some light on the cause of the high leucocyte content of apparently healthy cows.

The detailed results are herewith presented in Table 3 in which the daily observations are recorded in full, so that the same may be critically studied by other observers. These data can be comprehended in their entirety more readily, in graphical, rather than tabular, form, and in Fig. 1 they are therefore reproduced.

In Table 4 is condensed a summary of the foregoing results, in which the maximum and the minimum, as well as the average leucocyte content, is given; also the various observations are grouped as to the respective number of times in which the leucocyte content came within certain specified limits.

In discussing these results the herd will be separated into two sections:

1. Cows which show an entire freedom from all udder troubles, either past or present.

2. Cows showing upon careful physical examination a slight induration or otherwise abnormal condition of the udder, but no unusual condition as to character of their milk supply.

Of the 30 animals in the herd, only one (No. 3) at the time these examinations were made showed any symptoms of active udder

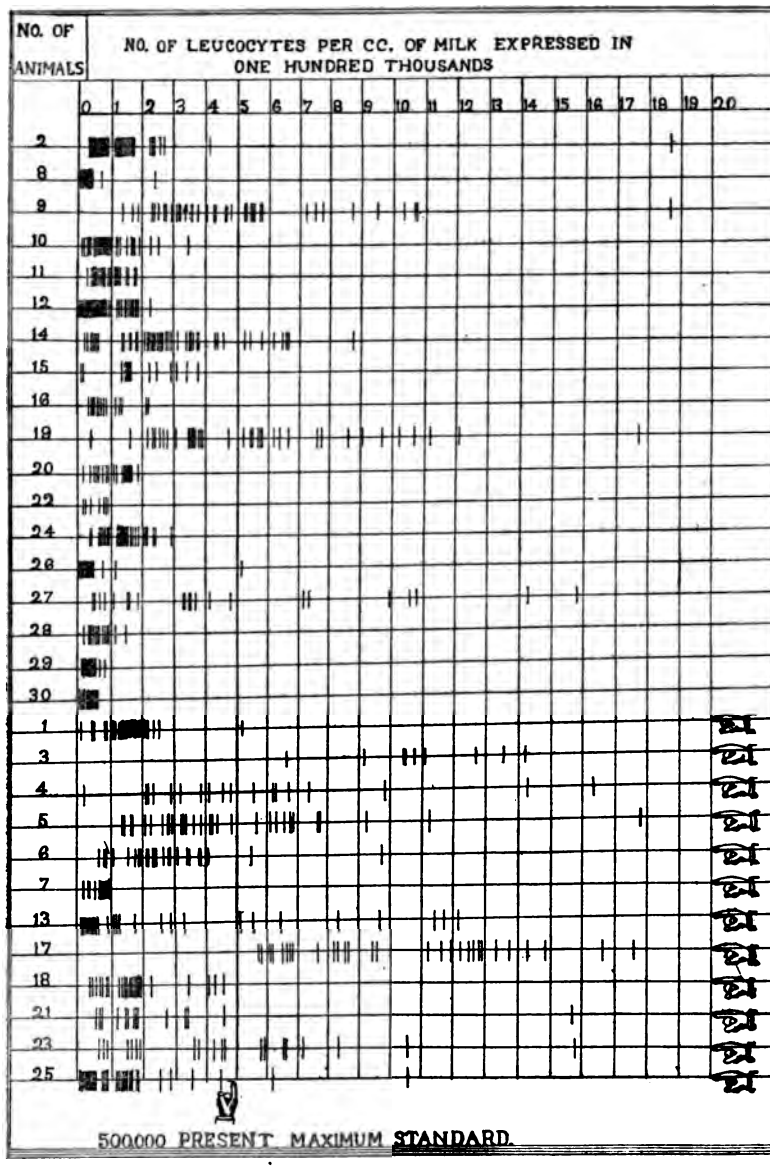

FIG. 1.—Leucocyte content of milks from University herd. Each determination represented by short vertical line. Animals marked with hand showed udder abnormalities. Note relative proportion of cases in excess of the present maximum standard.

trouble. In this case the milk was bloody and often clotted. The milk of the remaining 29 animals was perfectly normal in every respect, not only on the basis of its physical appearance, but by chemical and bacteriological analysis as well.

TABLE 4.

SUMMARY OF DATA IN TABLE 3.

Group 1. Perfectly Healthy Animals.

No.	Condition	No. of Tests	Maximum Count	Minimum Count	Average of All Tests	No. Times Leucocyte Content per c.c. Fell within following limits				
						50,000 or Below	From 50,000 to 100,000	From 100,000 to 500,000	From 500,000 to 1,000,000	1,000,000 and Above
2	Normal	38	403,750	26,250	121,175	8	10	20	..	
8	"	20	63,750	1,250	18,815	19	1	..	..	..
9	"	38	1,966,250	126,250	536,280	..	..	21	13	4
10	"	38	338,750	3,750	100,400	8	15	14	..	..
11	"	38	162,500	20,000	85,130	5	19	14	..	..
12	"	36	216,250	11,250	85,225	11	12	13	..	..
14	"	37	892,500	137,500	327,000	..	..	29	8	..
15	"	12	341,250	128,750	235,520	..	..	12	..	..
16	"	16	216,250	41,250	96,360	3	7	6	..	..
19	"	30	1,707,500	35,000	561,825	1	..	17	13	5
20	"	41	412,500	11,500	162,500	2	8	31	..	..
22	"	7	81,850	16,250	52,770	3	4	..	..	..
24	"	39	297,000	38,750	121,300	2	16	21	..	..
26	"	39	532,500	3,750	42,000	36	1	1	1	..
27	"	23	2,191,250	58,000	652,960	..	4	10	2	7
28	"	24	201,250	16,250	60,130	14	7	3	..	..
29	"	29	72,750	3,750	25,000	29	2	..	..	..
30	"	26	46,250	31,250	19,880	26	..	..	..	..
	Totals	537	.....	.....	.....	167	106	212	37	16
	Percentage of total	...	.....	.....	.....	31.1%	19.8%	39.4%	6.8%	2.9%

Group 2. Animals Having Udder Indurations of Varying Severity but Producing Apparently Normal Milk (Except No. 3).

1	Slight induration	38	538,750	11,250	167,725	1	7	29	1	..
3	Bad; chronic mammitis	27	3,625,000	16,250	903,380	5	3	6	2	11
4	Udder coarse and hard	19	4,952,500	21,300	972,700	1	..	8	6	4
5	Slight; one-quarter	39	1,781,250	122,500	476,520	..	..	27	10	2
6	Indiges.; normal otherwise	19	543,750	75,000	213,700	..	2	10	1	..
7	Slight; one-quarter	38	231,250	7,500	63,375	15	17	6	..	..
13	One-quarter harder	43	1,191,250	10,000	225,935	18	6	11	6	2
17	Slight; one-half of udder	40	4,232,500	150,000	1,274,115	..	..	8	13	19
18	One-quarter slight (?)	32	1,801,250	32,500	249,450	2	7	21	..	2
21	One-quarter harder	15	1,581,250	66,850	304,125	..	3	11	..	1
23	Slight in one-quarter	22	4,132,500	62,500	630,170	..	3	10	6	3
25	One-quarter affected	39	1,068,000	8,750	149,950	7	13	17	1	1
	Totals	371	.....	.....	.....	49	61	170	46	45
	Percentage of total	...	.....	.....	.....	13.2%	16.4%	45.9%	12.4%	12.1%

RESULTS.

A study of the preceding tabular data shows some peculiar results. It is apparent that the leucocyte content of some of the cows was remarkably uniform, while in a considerable number of cases the

results were widely variable. It is to be noted, however, that the more uniform results were found in the first section of the herd, in which no previous history of udder trouble was observed. Of the 18 out of the 29 animals which showed a leucocyte content that was quite constant on successive examination, 14 cases belonged to Group 1 and only four to Group 2. Of the 11 instances in which the leucocyte content was subject to wide fluctuations, only four belonged to Group 1 while seven were found in the second group in which there was a more or less marked induration in the udder.

This would seem to indicate that lower and more constant results are more likely to be obtained with animals in which no udder trouble had ever appeared than with those having any history of a past inflammatory condition.

It is of course hardly possible to average results that fluctuate so widely, and draw any satisfactory conclusions, but if the data are arranged, as in Table 4, on the basis of the number of times the leucocyte content fell into certain numerical groupings, it is evident that the cellular content of milks drawn from perfectly normal cows averages lower than in Group 2.

Doane has suggested 500,000 leucocytes per c.c. as a possible limit by his method. On the basis of this standard, the results of our examination would be as follows:

Eighteen animals in the perfectly healthy group showed in 537 tests a leucocyte content of 500,000 or less in 90 per cent of the cases, while the 11 animals, in which induration was more or less marked, in 371 tests showed a similar condition in 75 per cent. While it appears from this that milks of high leucocyte content were found with considerably greater frequency (approximately two and one-half times), in the case of cows showing a fibrous induration of the udder, still it is noteworthy that practically one-tenth of all examinations made on cows entirely normal and healthy in all respects showed a leucocyte content that was in excess of the highest standard yet established. In this group of perfectly healthy animals 16 cases (or 3 per cent) were observed in which the cellular content was in excess of even 1,000,000 cells per c.c.

In the second section of the herd two animals contributed 30 of the 45 observations in which more than 1,000,000 leucocytes per c.c. were found. One of these (No. 3) was the only animal in the herd

that showed any acute udder trouble. No. 17, while showing no marked involvement of the udder, almost invariably had a high leucocyte content.

When these results are studied on the basis of whether they would pass the standards that have been proposed as a maximum limit, it appears that in many cases they would have been condemned. While it is impossible to compare accurately the results of the examination by the direct quantitative method with what would have been found by the smeared-sediment method, it is certain that a considerably higher standard would be necessary, if the Doane-Buckley method was the one in general use.

It is apparent from these studies that the leucocyte content of normal milk drawn from apparently normal animals is quite often so high that the milk would be classed as coming from diseased animals when judged by the standards that have heretofore been proposed. That such results are obtained from animals whose record has shown no clinical history of a diseased condition would signify that complete reliance cannot be placed upon quantitative leucocyte standards alone.

While it is undoubtedly true that cows suffering from udder trouble often do produce milk that is rich in leucocytes, it would seem equally true that similar quantitative results were found with such frequency in milks from perfectly healthy animals as to vitiate the accuracy of a test based solely on a numerical foundation. This being true, it seems necessary to modify the limits that have been adopted by a number of cities where the acceptance or rejection of a milk supply is based upon such standards as are here considered. As interpreted at present, they undoubtedly work injustice to the dairy interests, as milk supplies would be frequently condemned which come from animals in which no clinically recognized troubles can be ascertained.

Undoubtedly the leucocyte standard can be so formulated as to be of material service in the matter of milk inspection, but the question needs further study. So far as technique is concerned, the volumetric method (Doane-Buckley) is the more accurate. As to its applicability in routine inspection work, our experience is that this test can be made as rapidly as the smeared-sediment method, and is less trying on the eyes.

THE COMPARATIVE VALUE OF BACTERIAL AND TEMPERATURE REGULATIONS FOR A CITY'S MILK SUPPLY.

FRANCIS H. SLACK.

(From the Boston Board of Health Laboratory, Boston, Mass.)

IN May, 1904, Boston established a bacterial regulation requiring market milk to contain not more than 500,000 bacteria to the c.c., and a temperature regulation requiring the milk to be kept below 50° F. Since these regulations were established simultaneously, it is impossible to give the effect of each singly in improving the milk supply; but a comparison of temperatures and bacterial counts on the same samples is instructive, and gives an insight into the relative values of the two regulations, each of which is shown to be important.

This paper is based on a total of 11,403 samples taken during three years, from June 1, 1904, to November 1, 1906. During this period both temperatures and plate counts were taken on 8,589 samples. On the remaining 2,814 samples temperatures were taken, but no counts were made, because they were passed as within the limit for bacteria on the microscopic examination alone; the error of this latter method with a careful worker is practically negligible as shown in a previous paper read before this Association.[1] (Of the 2,814 samples so passed 89 per cent were also within the temperature regulation.)

The condition of a milk supply untrammeled by bacterial or temperature regulations may perhaps be approximated by a consideration of the data of the first seven months' work in Boston after the regulations were made and while the work was educational in its character, warning notices alone being sent to those who failed to meet the conditions. The double examination, i. e., bacterial count and temperature, was made on 3,043 samples seized during these 7 months. The results of these examinations are as follows: 54¼ per cent were within both standards; 24¼ per cent had high temperatures with low bacterial content; 12 per cent had high bacterial content with low

[1] *Rep. and Papers, Amer. Pub. Health Assoc.*, 1905, 31, Pt. 2, p. 214.

temperatures and 9½ per cent were outside of both standards. Judging this milk from a temperature standpoint alone, 33¾ per cent would have been condemned instead of 21½ per cent. Of the milk thus condemned on temperature about 72 per cent would have been within the bacterial standard, while over half of the milk which was proven unfit for use by the bacterial examination was at a temperature below 50° F. when the samples were taken.

The second year, 1905, the previous work began to show results. Inspections were made constantly, warning notices sent as before, and a few cases were fined in court for continued failure to comply with either or both regulations. The samples examined for this second year then may fairly represent a milk supply where these regulations are in effect, and an honest effort is being made to produce a milk supply complying with them. We have results for both temperature and bacterial examination of 4,453 samples for 1905 as follows: 73 per cent were within both standards; 4 per cent had high temperatures with low bacterial content; 20 per cent had high bacterial content with low temperatures, and 3 per cent were outside both standards. Judging this milk from a temperature standpoint alone, 7 per cent would have been condemned instead of 23 per cent; 60 per cent of the milk thus condemned on temperature alone would have been within the bacterial standard and 87 per cent of the milk which bacterial examination showed unfit for use would have escaped notice. These results show a gain of about 20 per cent in milk within both standards and of 26 per cent on the temperature standard alone. The smallness of the gain (1½ per cent) in the amount of milk exceeding the bacterial standard may be explained by the fact that during 1905 over 1,000 samples were taken from stores and wagons, while during the first period of seven months samples were taken at the contractors' receiving stations only. Of samples taken at the receiving stations during this second year only 12¼ per cent had a count above 500,000 to the c.c. or 9¼ per cent less than the preceding year.

During the present year the same conditions prevail. Ice has however been very scarce, and there have been no prosecutions. Under these conditions the milk supply shows some deterioration. Nine hundred and twenty-four samples have been taken from stores

and wagons. Up to November 1, we have results for both temperature and bacteriological examination of 3,907 samples, of which 66¾ per cent were within both standards; 9½ per cent had high temperatures with low bacterial content; 19 per cent had high bacterial content with low temperatures, and 4¾ per cent were outside of both standards. Judged from the temperature standpoint alone 14¼ per cent would have been condemned instead of 23¾ per cent; 66 per cent of the milk thus condemned on temperature would have been within the bacterial standard, and 80 per cent of the milk which bacterial examination showed unfit for use was within the required temperature.

A summary of the samples on which both examinations were made for 29 months shows that of a total of 11,403 samples 66 per cent were within both standards; 11¼ per cent had high temperatures with low bacterial content; 17½ per cent had high bacterial content with low temperatures, and 5¼ per cent were outside both standards. Judged from the temperature standpoint alone, 16½ per cent would have been condemned instead of 22¾ per cent. Of the milk thus condemned on temperature, 68 per cent would have been within the bacterial standard, while 77 per cent of the milk which was proven unfit for use from high bacterial content would have escaped notice.

More striking still is a consideration of the relation of temperature and bacterial content at different periods of the year. July is our worst summer month. We have records of 1,153 samples taken during July in the three years; 30½ per cent of these were within both standards; 17¼ per cent had high temperatures with low bacterial content; 35¼ per cent had high bacterial content with low temperatures, and 17 per cent were outside of both standards. If judged by temperature alone 37 per cent would have been condemned instead of 52 per cent. About half of the milk thus condemned on temperature would have been of low bacterial content, while over two-thirds of the milk which bacteriological examination proved unfit for use would have escaped notice. December stands at the other extreme. Of the 552 December samples on which we have records of both temperature and count, 89½ per cent were within both standards; 3¾ per cent had high temperatures with low bacterial content; 6¾ per cent had high bacterial content with low temperatures, and there were

TABLE 1.

COMPARISON OF TEMPERATURE AND COUNT ON SAMPLES TAKEN DURING 29 MONTHS.

Year	Temperature Low Count Low			Temperature High Count Low			Temperature Low Count High			Temperature High Count High		
	1904	1905	1906	1904	1905	1906	1904	1905	1906	1904	1905	1906
January	...	162	491	...	1	42	...	16	51	...	0	7
February	...	502	369	...	8	31	...	39	24	...	2	1
March	...	459	348	...	17	35	...	78	32	...	1	1
April	...	325	451	...	23	14	...	66	43	...	0	4
May	...	345	111	...	3	11	...	38	126	...	2	8
June	192	346	62	60	58	12	37	92	116	36	41	38
July	209	101	42	181	13	4	103	197	107	111	31	54
August	215	87	212	264	16	70	52	116	72	90	18	28
September	349	40	198	117	4	67	110	114	64	35	21	18
October	252	285	327	45	39	83	27	72	106	11	8	27
November	286	256	...	53	0	..	29	83	...	5	0	..
December	149	345	...	18	2	..	7	31	...	0	0	..
	1,652	3,253	2,611	738	184	369	365	892	741	288	124	186

TABLE 2.

SAME AS TABLE 1, EXCEPT THAT FIGURES FROM DIFFERENT YEARS IN THE SAME MONTH ARE ADDED.

	Temperature Low Count Low	Temperature High Count Low	Temperature Low Count High	Temperature High Count High	Totals
January	653	43	67	7	770
February	871	39	63	3	976
March	807	52	110	2	971
April	776	37	109	4	926
May	456	14	164	10	644
June	600	130	245	115	1,090
July	352	198	407	196	1,153
August	514	350	240	136	1,240
September	587	188	288	74	1,137
October	864	167	205	46	1,282
November	542	53	62	5	662
December	494	20	38	0	552
Totals	7,516	1,291	1,998	598	11,403

TABLE 3.

SAME AS PREVIOUS TABLE BUT EXPRESSED IN PERCENTAGE.

	Temperature Low Count Low	Temperature High Count Low	Temperature Low Count High	Temperature High Count High
January	84.80%	5.59%	8.70%	0.91%
February	89.24	4.00	6.45	0.31
March	83.12	5.35	11.33	0.21
April	83.80	4.00	11.77	0.43
May	70.82	2.17	25.46	1.55
June	55.05	11.92	22.48	10.55
July	30.53	17.17	35.30	17.00
August	41.45	28.23	19.35	10.97
September	51.63	16.54	25.33	6.50
October	67.39	13.03	16.00	3.58
November	81.87	8.01	9.37	0.75
December	89.50	3.62	6.88	0.00
Totals	65.91%	11.32%	17.52%	5.25%

TABLE 4.

SHOWING COMPARISON OF TEMPERATURE AND BACTERIAL CONTENT FOR THE DIFFERENT YEARS.

	Temperature Low Count Low	Temperature High Count Low	Temperature Low Count High	Temperature High Count High	Totals
June 1 to Dec. 31, '04.....	1,652 or 54.29%	738 or 24.25%	365 or 12.00%	288 or 9.46%	3,043
Jan. 1 to Dec. 31, '05.....	3,253 or 73.05	184 or 4.13	892 or 20.03	124 or 2.79	4,453
Jan. 1 to Oct. 31, '06.....	2,611 or 66.83	369 or 9.44	741 or 18.97	186 or 4.76	3,907

no samples outside of both standards. If judged by temperature alone, 3¾ per cent would have been condemned instead of 6¾ per cent. All the milk thus condemned on temperature would have been good

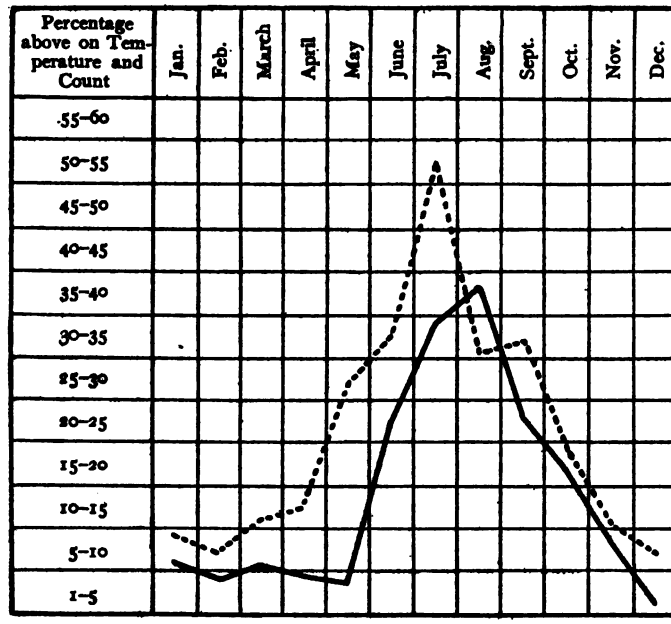

CHART 1.

COMPARISON OF TEMPERATURE AND COUNT.

This diagram shows average high temperature (plain line) and high count (dotted line) milk for 29 months, June 1, 1904, to November 1, 1906. While they follow the same curve almost exactly, the high count milk is for the most part low temperature, and vice versa, as shown by the preceding tables. Such a chart as this is apt to be quite misleading if all the factors are not stated.

as to bacterial content, while all the milk which the bacterial examination proved unfit for use would have escaped notice.

In attempting comparison of these two tests it is necessary first to point out the relative scope of each. The dealer whose milk com-

plies with the *bacterial* standard must, we believe, have good cows, well housed and tended, must handle his milk in a cleanly manner, must cool his milk, and transmit it without delay to the consumer. These four points, emphasized many times heretofore, are the essential factors in producing good clean milk. It is evident that the temperature standard forces the observance of but one of the factors, cold, while the bacterial standard forces the observance of all four.

Unquestionably, the temperature factor is an important one in the production of good milk, especially in hot weather, since the condemnation of milk on the temperature standard alone, if widely applied at all stages of the progress of the milk from producer to consumer, would undoubtedly result in a very general observance of the temperature regulation. It can, however, never accomplish what the bacterial standard accomplishes.

The temperature test has the advantage of being less arduous, since the temperature of many samples may be taken with less labor than that required for one bacterial examination, but it is probably true that in a large city it is of comparatively little importance whether 20 or 100 samples are examined in a day, so long as samples are seized every day without notice.

If either test is to be chosen, to the entire exclusion of the other, the count is undoubtedly the one to choose, since to keep milk low in bacterial count would necessitate its being kept cold.

COMPARISON BETWEEN BACTERIOLOGICAL ANALYSIS OF AIR BY THE PLATE METHOD AND BY FILTERS.

GEORGE A. SOPER.

Two principal methods have so far been used by investigators to determine the numbers of bacteria in air: First, Petri dishes, containing a suitable culture medium, have been exposed for the organisms to settle upon during a definite period of time; and, second, the bacteria have been collected by means of filters through which a measured volume of air has been passed.

An opportunity was afforded me during a recent investigation of the air of the New York subway to compare the serviceability of these two methods on a scale and under conditions which were capable of giving a useful idea of their relative value.

The determinations by the plate method numbered about 2,800; the determinations by the filter method, 166. The work was all done in duplicate. The air examined was the air of the New York subway and the air of the streets. As far as practicable, the observations by the two methods were made under the same circumstances as to hour and place, but they were not made simultaneously.

THE PLATE METHOD.

The plates used were Petri dishes about 3½ inches in diameter. The culture-medium was beef-extract agar. The reaction, determined by preliminary experiments to ascertain the optimum, was 1 to 1.5 per cent acid to phenolphthalein.

It was customary to pour the agar into the plates at the laboratory and incubate them for twelve hours at 37° C. before exposing them to detect accidental contamination. The plates were carried from the laboratory to the point where the air was to be examined, wrapped in sterile towels, and fitted closely into a handbag. The period of exposure was 15 minutes in those cases where the numbers of bacteria were not expected to be large.

After exposure, the plates were taken to the laboratory, incubated at 37° C., and counted after 48 hours. Moulds were distinguished from the bacteria and a separate record kept of their number.

THE FILTER METHOD.

The filter method was substantially that used by Sedgwick, Prudden, and others, with some modifications. The filters were glass tubes about 0.5 c.c. inside diameter, and 13 cm. long. The filtering material was held in place by a plug of wire gauze at one end and at the other freely exposed to the air. During transportation a plug of sterilized cotton closed the tube at each end.

After preliminary trials of various filtering materials, including some soluble ones, the medium finally adopted was sand. The depth of sand was about 5 cm. The particles were chiefly quartz. The grains ranged from about 1 mm. in diameter down to very fine particles. Two filters were always arranged in tandem.

Air was passed through the filters in most cases by means of a carefully constructed air pump. The quantity of air was determined by the number of strokes of the pump. To pump 20 liters of air through the filters, 66 strokes were required. This was the amount generally used in each case.

It was sometimes not feasible to use an air pump, where its use would attract a crowd of curious people. Under these circumstances, a brass vacuum cylinder of about 10 liters capacity, fitted with a pressure gauge and suitable stop cocks, as devised by Prudden, was employed. This cylinder was fitted into an inconspicuous leather handbag. Through suitable openings the gauge could be seen and the stop-cocks operated. Before taking a sample, the air was exhausted from the cylinder and the stop-cocks closed. The apparatus was then carried to the place where the observation was to be made. The filters were there connected to the cylinder by means of short rubber tubes and, after reading the gauge, the air was allowed to flow into the cylinder through the filters. The cocks were closed when the desired quantity of air had been filtered, as determined by a second reading of the gauge. Finally, the filters were replugged and taken to the laboratory.

At the laboratory the sand was emptied from the first filter into a test-tube which contained 10 c.c. of sterile water. After thoroughly agitating the sand and water, the organisms which were rinsed from the sand were plated in agar of similar composition to that already described as having been used in the filter method. The agar was

incubated for 48 hours at 37° C. and the colonies counted, moulds being recorded separately.

Most of the organisms were caught in the first filter. The percentage which those in the second filter bore to those in the first, as determined by 140 analyses, was 2.6.

RESULTS AND CONCLUSIONS.

As determined by the plate method and by the filter method, the same relation appeared to exist between the bacteria in the air of the subway and in the streets. The average numbers of bacteria which settled from the air in 15 minutes and were subsequently enumerated by the plate method were, in the subway, 500; in the streets, 1,157; ratio 1 to 2.3. The average numbers of bacteria found by the filter method were, in the subway, 3,200 per cubic meter of air; in the streets, 6,500; ratio 1 to 2.0.

This interesting relation between the results of examinations of the air of the subway and streets by the two methods should not be taken to indicate that both methods were accurate, for, as is well known, there is no precise way to determine the numbers of bacteria in air. The two methods were the most accurate which it was found feasible to employ upon the scale required. The plate method was very much more convenient than the filter method, and gave results which in this case were sufficiently instructive to warrant its almost exclusive employment as a general routine procedure. The filter method, although it probably gave a more accurate idea of the condition of the air, involved more difficulties of technique than the superiority of its results warranted. Together the two methods yielded data from which a large number of profitable deductions were drawn.

THE AGGLUTINATION METHOD OF DIAGNOSIS IN THE CONTROL OF GLANDERS

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IN the control of glanders the first and most important requirement is an accurate diagnosis. The inoculation method of Strauss gave much assistance in cases of apparent lesions, from which inoculating material could be obtained. This, however, did not afford assistance in cases of horses which had been exposed to the disease, but which did not exhibit symptoms or lesions of the malady. Mallein was welcomed as a means of detecting the disease in cases of this kind and in obscure cases generally, but its application has not been altogether satisfactory. Its limitations have not been so clearly defined as those of tuberculin, and the obstacles and complications in its use render many of the results difficult of interpretation. For these reasons a method that can be applied by a trained man, eliminating nearly if not all of the troublesome features of the mallein test, is worthy of careful consideration.

The agglutination method of diagnosis, which was first applied to glanders by M'Fadyean, was recommended in the work of control of the disease by Schütz and Miessner, and by Schnürer, and more recently studied in this laboratory, offers a possible solution for several of the difficult features in the diagnosis of glanders.

The method as formulated by Schütz and Miessner has been officially adopted for the diagnosis of glanders generally in Prussia. When there are cases of suspected glanders, the veterinarian or official in charge applies for sterile bottles, which are forwarded from the laboratory to receive the blood. The specimen of blood and the horse from which it was taken are given a number for identification, and the samples are sent to the laboratory. The test is made and the results reported, with directions for the disposition of the animal.

In Austria, Schnürer makes the diagnosis of glanders in suspected cases for the Austrian army. His method of procedure is practically the same as that of Schütz and Miessner, and he reports it as more

accurate than any other method for the diagnosis of this disease. During the last year, Moore, Taylor, and Giltner have tested the method very carefully, using it in the diagnosis of a considerable number of cases of suspected glanders with very satisfactory results. Dr. Berns, a veterinary practitioner of Brooklyn, N. Y., has used it for the last six months in his practice, and reports the most satisfactory results from it in 170 horses to which he had applied it at the time of writing.

The development of the method can be followed from the writings of M'Fadyean, Bourget and Méry, Arpàd, Fedorowsky, Rabieaux, Reinecke, and Bonome.

Schütz and Miessner recommend the use of glanders bacteria that have been killed by heating them for two hours at 60° C. The killed organisms were suspended in a carbolized-salt solution. The suspension was made of a light-greyish color and distributed in small test tubes, 2 c.c. in each. Various quantities of the diluted blood-serum were added to this emulsion, and the mixture incubated for 24–30 hours at 37° C.

The results of these experiments demonstrated that while the blood-serum of healthy horses agglutinated the bacteria in dilutions of 1–400 at the highest, the serum of glandered horses reacted in dilutions of from 1–1,000 to 1–2,000. The blood-serum of horses artificially infected with a virulent culture gave a reaction beginning on the fifth to the seventh day after inoculation, the agglutinating power increasing during the following four of five days, remaining at its maximum for about one month, and after that gradually diminishing. This is of practical importance in detecting glanders in a stable where infected horses have been destroyed, the test being made two or three weeks later. Experience led to the belief that a previous malleination had no effect on the agglutinating power of the serum.

The blood of non-glandered but diseased horses reacted occasionally in higher dilutions. Thus it is found in cases of pleurisy and pneumonia that a reaction occurred in a dilution of 1–1,000, while in other cases it did not occur in dilutions higher than 1–800. In the Pathological Institute in the Veterinary College in Berlin, during two years, the blood-serum of 2,209 horses was tested for glanders with the following results:

Of 1,911 horses free from glanders; in

1,232	or	64.8	per cent	the	blood-serum	agglutinated	1–100 to 1–300
363	"	19.0	"	"	"	"	1–400
135	"	7.1	"	"	"	"	1–500
123	"	6.4	"	"	"	"	1–600
41	"	2.2	"	"	"	"	1–800
11	"	0.5	"	"	"	"	1–1,000

A reaction higher than 1–1,000 was not observed in a single case.

Of 298 glandered horses; in

6	or	2.0	per cent	the	blood-serum	agglutinated	at 1–400
12	"	4.0	"	"	"	"	" 1–500
44	"	14.8	"	"	"	"	" 1–600
47	"	15.8	"	"	"	"	" 1–800
75	"	25.2	"	"	"	"	" 1–1,000
49	"	16.4	"	"	"	"	" 1–1,500
65	"	21.8	"	"	"	"	" 1–2,000

A study of these tables shows that the greater percentage of reactions with normal serum has been with very low dilutions, while the greater percentage with glandered serum has reacted in dilutions so high that they were positively diagnostic. Experience shows that in glandered horses the agglutinating power of the blood is with the passing of time gradually diminished, while in horses free from glanders the agglutinating power of the blood does not change. Based upon their experience, Schütz and Miessner recommend the following method for the eradication of glanders:

Twenty to 50 grams of blood are taken from the glandered or suspected horse, the date and history of the case being recorded and sent to the experiment station.

All horses whose blood agglutinates in dilutions of 1–1,000 or higher should be destroyed.

In the same way, all horses should be destroyed whose blood agglutinates in dilutions of only 1–500 to 1–800, if they show symptoms of glanders.

All other horses in which the agglutination is 1–500 to 1–800 should be isolated and destroyed only when justified by a second test, in which the maximum dilution for agglutination is changed; on the other hand, they may be pronounced free from glanders if at the second test the dilution remains unchanged.

After glanders is established, the blood of horses in the same stable should be tested after three weeks; and this should be repeated until the last two tests show in all horses individually a uniform reaction.

In our work we have applied the general method recommended by Schütz and Miessner, with such slight modifications as seemed desirable. The method for the routine diagnosis which we have found to be very satisfactory is as follows:

Culture.—As pointed out by Schütz and Miessner, all cultures of *B. mallei* do do not agglutinate satisfactorily. It was also shown by their work that a suitable culture when obtained is liable, at unexpected intervals, to lose its responsiveness to the agglutinin. This can be prevented by passing the organisms through a guinea-pig at least once in three weeks. The organisms were grown for from 48 to 72 hours

on acid-glycerin agar (5 per cent glycerin and with a reaction of +2.9 to phenolphthalein). In order to have a suitable culture on hand, subcultures should be made daily. A culture more than 72 hours old should not be used in preparing the test fluid.

Test-fluid.—The test-fluid is prepared by washing the growth from the agar culture by the aid of a sterile wire loop into distilled water containing 0.85 per cent sodium chloride and 0.5 per cent carbolic-acid crystals. This suspension is then placed in a thermostat at 60° C. for two hours, which kills the bacteria. A temperature higher than 65° C. or lower than 60° C. should be avoided. After heating, the suspension is thoroughly triturated and filtered through sterile cotton. Thorough trituration of the emulsified growth is essential before filtering. The filtrate thus prepared is diluted with the carbolized-salt solution until it is of a faintly cloudy appearance. The proper dilution of the filtrate can only be determined by experience. The test-fluid gives the best results when made with freshly prepared carbolized-salt solutions.

Procuring the serum.—The serum is easily obtained. At least 10 c.c. of blood are drawn from the jugular vein, under aseptic precautions, into a small sterile bottle, and sent to the laboratory. As soon as the clot forms, the supernatant serum is placed in a centrifuge and all the sediment thrown down, leaving the liquid perfectly clear. One c.c. of the serum is then added to 39 c.c. of a physiological salt solution, which makes a dilution of 1–40. It is desirable that the serum should be secured as soon as possible after the blood is drawn. If necessary to delay the test, the serum has given the best results if kept at about 10° C. until used. The diluted serum tends to deteriorate if kept more than 24 to 48 hours. Even during this time it should be kept at a low temperature. Serum that has decomposed should not be used.

Making the test.—Three c.c. of the "test-fluid" are placed in each of several small test-tubes. With a sterile pipette, the diluted serum is added to the tubes of test-fluid and thoroughly mixed. In making the different dilutions, the amount of diluted serum to be used is readily ascertained by the following table:

TABLE 1.

Dilution of Serum	Amount of Diluted Serum	Amount of Test-Fluid	Dilution
1–40	1.2 c.c.	3 c.c.	1–100
1–40	0.6	3	1–200
1–40	0.405	3	1–300
1–40	0.3	3	1–400
1–40	0.24	3	1–500
1–40	0.195	3	1–600
1–40	0.15	3	1–800
1–40	0.12	3	1–1,000
1–40	0.105	3	1–1,200
1–40	0.09	3	1–1,500
1–40	0.06	3	1–2,000
1–40	0.03	3	1–4,000
1–40	0.015	3	1–8,000

Where dilutions greater than 1–1,000 are made, a serum diluted 1–80 may be used to better advantage, unless the pipette employed is very finely graduated. In this case the amount of diluted serum for a certain dilution must be double that indicated in the table.

The mixture thus prepared is placed in an incubator at 37° C. for 24–30 hours. A temperature higher than 37° C. interferes with the agglutination.

Reaction.—The reaction consists of a layer of the agglutinated bacteria covering the entire convexity at the bottom of the tube. This film-like sediment may become so dense that it rolls in at the periphery. The supernatant fluid becomes clear in the lower dilutions, but in the higher ones the clarification may not be complete, showing that all the bacteria have not become agglutinated. This is further evinced by the fact that the layer is less dense in the higher dilutions. The reaction may begin in six hours, but cannot be considered complete until 24 to 26 hours have elapsed. If no reaction appears in 24 hours it cannot be considered negative, as it may occur in from 30 to 40 hours after setting. Often, however, a reaction appears in less than 24 hours.

After the agglutination is completed, further standing produces no visible change in the test-fluid.

A negative result shows a small round concentrated spot of sediment in the center of the convexity at the bottom of the tube, the test-fluid remaining apparently unchanged even after several weeks.

In our examinations, we have confined our work very largely to the macroscopic appearances. It is believed, however, that not infrequently helpful information could be obtained by a microscopic examination as well. However, we have not found it safe to depend on a microscopic reaction for diagnosis, because of the variability of horse sera in agglutinating *B. mallei.* Often the microscope will reveal the presence of clumping, when macroscopically it cannot be detected. Our experience has led to the belief that a high dilution microscopic reaction cannot be considered diagnostic. The macroscopic precipitation of the agglutinated bacteria seems to be essential before drawing conclusions. In testing suspected blood, we have followed the plan of making for each examination dilutions of 1–200, 1–500, 1–800, 1–1,000 and 1–1,200. If a reaction took place at 1–1200 higher dilutions were tested. By this method we were able to tell if the culture were reliable by observing the reaction at the dilution of 1–200 as this should agglutinate even with non-glandered serum. If a reaction occurred in the absence of symptoms at 1–800, the case was considered suspicious and retested in from a few days to three weeks later. If a reaction appeared at 1–1,000, 1–1,200 or higher, the animal was considered glandered.

Thus far, as shown in Tables 1 and 2, we have not had a reaction with the serum from a non-glandered horse above 1–500. The majority failed to react above 1–400. In all cases where we have had a reaction of 1–1,000 or higher, the animal has shown conclusive clinical evidence of glanders, or upon postmortem examination has

exhibited characteristic lesions of that disease. This corresponds to the findings of Schütz and Miessner.

We have applied this test to the blood-serum of a total of 81 horses. Some of these were in good health; some were suffering from diseases other than glanders; still others, and by far the largest number, were believed to be glandered or suspected of having the disease because of certain symptoms; or they appeared to be healthy but had been exposed. A summary of the percentages of the maximum dilutions of the serum at which agglutination occurred is appended:

Of 19 healthy horses; in

3	or	15.8	per	cent	the	maximum	dilution	was 1–200
2	"	10.5	"	"	"	"	"	" 1–300
11	"	57.9	"	"	"	"	"	" 1–400
3	"	15.8	"	"	"	"	"	" 1–500

Of 12 diseased but not glandered horses; in

1	or	8.3	per	cent	the	maximum	dilution	was 1–200
5	"	41.7	"	"	"	"	"	" 1–300
4	"	33.3	"	"	"	"	"	" 1–400
2	"	16.7	"	"	"	"	"	" 1–500

Of 50 horses suspected of having glanders; in

1	or	2	per	cent	the	maximum	dilution	was 1–3,200
1	"	2	"	"	"	"	"	" 1–2,800
1	"	2	"	"	"	"	"	" 1–2,000
7	"	14	"	"	"	"	"	" 1–1,600
14	"	28	"	"	"	"	"	" 1–1,500
12	"	24	"	"	"	"	"	" 1–1,400
4	"	8	"	"	"	"	"	" 1–1,200
1	"	2	"	"	"	"	"	" 1–1,000
1	"	2	"	"	"	"	"	" 1–800
8	"	16	"	"	"	"	"	" 1–500 or less.

The dilutions in which agglutination occurred in the serum of each of 68 horses including the three classes of cases are to be found in Tables 2, 3, and 4.

The clinical history of all the cases recorded in Table 4 as reported by the veterinarians who had them in charge shows that in every case in which the macroscopic agglutination occurred in the maximum dilution of 1–500 or lower the horse did not have glanders, and that in every case in which agglutination occurred at dilutions of 1–1000 or higher the horse was suffering from some form of glanders.

The delicacy of the method is illustrated in case No. 11. The horse had been exposed, but showed at the time the blood was taken

no evidence of the disease. Three weeks later it developed numerous typical lesions. Again in No. 15 the horse exhibited suspicious symptoms, but it was thought by some veterinarians not to be glandered. After the test the horse was condemned, as it gave a positive reaction to mallein, and on postmortem was found to contain typical glanders lesions.

In two cases, not reported in this table, the agglutination occurred with a maximum dilution of 1–400 and 1–500 respectively. These we reported as negative; but the veterinarians in charge replied that the horses were glandered and had been destroyed. Unfortunately they were not examined postmortem.

It has already been pointed out by others that the agglutinating power of the serum diminishes quite rapidly after the disease becomes established. We have not had an opportunity to test this phase of the reaction.

TABLE 2.

AGGLUTINATION OF *B. mallei* WITH SERUM FROM HEALTHY HORSES.

Number	1–100	1–200	1–300	1–400	1–500	1–600	1–700	1–800	1–900
1	+	+	+	+					
2	+	+	+	+					
3	+	+	+	+					
4	+	+	+	+					
5	+	+	+	+					
6	+	+							
7	+	+							
8	+	+							
9	+	+	+						
10	+	+	+	+					
11	+	+	+	+	+				
12	+	+	+	+					
13	+	+	+	+					
14	+	+	+						
15	+	+	+	+					
16	+	+	+	+					
17	+	+	+	+	+				
18	+	+	+	+	+				
19	+	+	+	+					

TABLE 3.

AGGLUTINATION OF *B. mallei* WITH SERUM OF DISEASED BUT NOT GLANDERED HORSES.

Disease	1–100	1–200	1–300	1–400	1–500
Edema of nasal septum	+	+	+	+	
Acute skin eruption	+	+	+	+	+
Azoturia	+	+	+		
Azoturia	+	+	+		
Azoturia	+	+			
Influenza, temperature 105.3°	+	+	+		
Pneumonia, temperature 102.3°	+	+	+	+	
Typhoid pneumonia, temperature 106.3°	+	+	+	+	+
Typhoid pneumonia, temperature 103°	+	+	+		
Acute laminitis	+	+	+	+	
Acute influenza, temperature 106.2°	+	+	+	+	
Distemper	+	+	+		

TABLE 4.

AGGLUTINATION OF *B. mallei* WITH THE SERUM OF HORSES SUFFERING WITH OR SUSPECTED OF HAVING GLANDERS.

No.	1-200	1-300	1-400	1-500	1-600	1-800	1-1000	1-1200	1-1400	1-1500	1-1600	1-1800	1-2000	1-2200	1-2400	1-2500	1-2600	1-2700	1-2800	1-2900	1-3000	1-3100	1-3200
1	+	+	+	+	+	+	+	+	+	+	+												
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+												
4	+	+	+	+	+	+	+	+	+	+	+												
5	+	+	+	+	+	+	+	+	+														
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7	+	+	+	+	+	+	+	+	+														
8	+	+	+	+	+	+	+	+	+	+	+												
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34	+	+	+	+	+	+	+	+	+	+													
35	+	+	+	+	+	+	+	+	+														
36	+	+	+	+	+	+	+	+	+														
37	+	+	+	+	+	+	+	+	+	+													

Berns and Way report the application of this method during the summer of 1906 in Dr. Berns's hospital. The following quotation from their paper is of interest:

> While the agglutination test is still in its infancy and our experiments are by no means completed, and our cases not sufficient in number to warrant positive conclusions, we are of the opinion that this method is a most valuable aid in diagnosing glanders, and from the above it would seem that this test not only reveals the presence of infection, but, to a certain extent at least, the degree of infection, by the reaction being either prompt or tardy, strong or weak, and the agglutination taking place in varying proportions from 1–200 up to 1–1,200 or even higher.

The application of the serum diagnosis of glanders in state and city sanitary work appears to have many advantages over the methods heretofore employed. The blood can be drawn by any veterinarian from the suspected horse with very little trouble and sent to the laboratory. However, the fact should be kept in mind that the method

is a laboratory test. Its requirements are such that it cannot be made in any bacteriological laboratory without sufficient notice and preparation. The necessary solutions must be ready, and cultures of *B. mallei* which are readily agglutinated must be in stock and of the proper age. To keep these in hand, requires more time, labor, and expense than can be given by laboratory men for an occasional diagnosis. The practical work, therefore, will be restricted to boards of health laboratories, or those doing the sanitary work for the city or state, and perhaps to those of practitioners who have a large practice in stables where cases of glanders are of common occurrence. As in Prussia and Austria, it would seem advisable for each large city and possibly state to have at least one laboratory where this work could be done from these sterile bottles for collecting the blood, and instructions could easily be sent to any veterinarian who wished to have the diagnosis made.

CONCLUSIONS.

From the results we have obtained in testing the various procedures in the serum diagnosis of glanders as set forth by various investigators and summarized in the preceding pages, the following conclusions seem to be warranted:

1. The diagnosis of glanders by the agglutination method is easier and quite as accurate as by mallein. It has this advantage, that it can be used in those cases where there is a rise of temperature, and consequently where mallein could not be employed.

2. There appear to be no objections to the recommendations of Schütz and Miessner for the eradication of glanders based on this method of diagnosis.

3. The maximum dilution of normal serum that we have found capable of producing macroscopic agglutination is 1–500. This is higher than that reported by others. It occurs, however, in but very few cases.

4. The maximum agglutinating dilution of the serum of diseased horses not glandered has not exceeded that of normal serum. This is lower than that recorded by others. We recognize, however, that our experience has been quite limited.

5. The interpretation of the results where the maximum dilution

is about 1–500 gives the greatest difficulty. All cases of this kind, unless there are unquestioned diagnostic symptoms or lesions, should be retained for a subsequent test.

6. The method, while simple in its details, requires in its application the closest of attention and constant checking, because of the liability of the culture losing unexpectedly its susceptibility to the agglutinins.

REFERENCES.

1. ARPÀD. "Beitrag zur Agglutination der Rotzbacillen," *Veterinarins*, 8 (ungarisch). Abs. by Hutyra in *Jahresbericht* for 1903, p. 34.
2. BERNS AND WAY. "Practical Application and Results of the Agglutination Method of Diagnosing Glanders in One Hundred and Fifty-two Cases," *Amer. Veter. Rev.*, 1906, 30, p. 822.
3. BONOME. "Ueber die Schwankungen des Agglutinin und Präzipitingehaltes des Blutes während der Rotzinfektion," *Centralbl. f. Bakt.*, etc., 1905, 38, p. 601.
4. BOURGET ET MÉRY. "Sur le séradiagnostic de la morve," *La semaine med.*, 1898, p. 61.
5. FEDOROWSKY. *Zur Frage über die Agglutination der Rotzbacillen vom vergleichend pathologischen u. differential diagnostischen Standpunkt aus betrachtet.* Dissertation. Petersburg. Abs. by Happisch in *Jahresbericht* for 1903, p. 34.
6. KLEINE. "Ueber Rotz," *Ztschr. f. Hyg.*, 1903, 44, p. 183.
7. M'FADYEAN. "Preliminary Note on the Sero-Diagnosis of Glanders," *Jour. Comp. Pathol. and Thera.*, 1896, 9, p. 322.
8. MOORE, TAYLOR AND GILTNER. "The Agglutination Method for the Diagnosis of Glanders," *Amer. Veter. Rev.*, 1906, 30, p. 803.
9. RABIEUX. "The Sero Diagnosis of Glanders," *Jour. de méd. Vétér.*, August, 1902. Abs. by M'Fadyean. *Jour. Comp. Pathol. and Thera.*, 1903, 16, p. 59.
10. REINECKE. "Die Sero-Diagnostik unter besonderer Berücksichtigung der Rotzkrankheit des Pferdes," *Ztschr. f. Veter.* 1904, p. 245.
11. SCHNÜRER. "Zur diagnostischen Verwertung der Rotzagglutination," *Centralbl. f. Bakt.*, 1905, 39, p. 180.
12. SCHÜTZ AND MIESSNER. "Zur Serodiagnose der Rotzkrankheit," *Archiv f. wis. u. Tierheilk.*, 1905, 31, p. 353.

THE GROWTH AND TOXIN PRODUCTION OF *BACILLUS DIPHTHERIAE* UPON PROTEID-FREE MEDIA.

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OF all the problems which fall within the bounds of bacteriology today, one of the most important is that which concerns the production of toxins by certain pathogenic micro-organisms. The toxin of diphtheria has, no doubt, so far as its chemical nature and conditions of formation are concerned, received the greatest amount of study from investigators. It presents, moreover, a problem in the biochemistry of bacteria, than which, to the present time, no other has yielded more satisfactory results, considered both in their relation to pure science and to their practical application in the treatment and prevention of disease. And yet there are many points in regard to the ultimate nature of these toxic products, their relation to and interaction with the antitoxins, which, to say the least, are very far from being well understood. Furthermore, it is probable that these elements of the problem must remain a matter of speculation only, until a more adequate knowledge shall have been gained regarding the chemical nature of these toxic products, and the conditions which are either favorable or necessary to their formation.

Although, in the qualitative analysis of the toxic substance produced by *B. diphtheriae*, the investigator can recognize certain organic or inorganic bodies, he is still in the dark with reference to the ultimate proteid body, whose exact constitution he wishes to determine. It is difficult to determine whether the albumoses are the toxic products, or whether the true toxin may not, in some way, be associated with them. Some investigators assume that the toxin itself may be an enzyme which has a proteolytic action and produces the virulent, but secondary, toxic products in the body of the animal. Whatever answer may be given to these diverse and often contradictory views, the fact remains that the diphtheria organism, growing upon a proteid-free medium, can synthesize a toxic substance of a proteid nature; and that the toxin is probably not (as previously claimed by Brieger

and Fraenkel) a toxalbumen resulting from the splitting up of proteid bodies within the medium.

Among the first to determine that the toxin of *B. diphtheriae* could be formed in a proteid-free medium was Uschinsky.[1] Since his discovery, however, reports have appeared, from time to time, stating that success has not been met with in growing the diphtheria organism upon proteid-free media. In reply to some of these criticisms, Uschinsky says:[2]

> Ich gebe zu dass auch ich viele Misserfolge gehabt habe. Jetzt aber besitze ich eine Kultur, welche auf meine Flüssigkeit vorzüglich wächst, und weit mehr Toxin produziert, als es bei mir früher der Fall war. Selbst 1½ c.cm. einer 4–6 Wochen alten filtrierten Kultur tödten sicher ein mittleres Meerschweinchen in 40–36 Stunden mit allen für Diphtherietoxin charakteristischen Erscheinungen.

Regarding the frequency of the growth upon his medium, Uschinsky continues:

> Die Ursachen meiner und anderer Misserfolge scheinen mir darin zu bestehen, dass wirklich nicht eine jede Diphtheriekultur zum Wachsen auf eiweissfreier Nahrlösung geeignet ist. Junge, frisch vom Menschen genommene Kulturen sind schwer auf dieser Lösung zu kultivieren; wogegen ältere, an saprophytische Lebensweise gewöhnte Kulturen leicht wachsen.

In the experiments about to be recorded, the writer has repeated Uschinsky's experiment, and has tried certain modifications of his medium together with some entirely new combinations. In all cases it was taken for granted that toxin-formations could not occur without at least a fair appearance of growth. With this fact in mind, the first aim of the writer was to obtain the best possible growth upon proteid-free media. Although in the first set of preliminary experiments the reaction of the media was adjusted to +0.45, as recommended by Hitchens,[3] in the later work (including all the combinations of media herewith presented), the reaction was made slightly alkaline, as determined by litmus.

USCHINSKY'S MEDIUM.

The first proteid-free medium to be tried was that recommended by Uschinsky. This was made up as follows:

[1] Uschinsky, *Centralbl. f. Bakt.*, 1893, 14, p. 316.
[2] Uschinsky, *Centralbl. f. Bakt.*, 1897, 21, p. 146.
[3] Hitchens, *Jour. Med. Res.*, 1905, 13, p. 523.

Water	1,000	parts
Glycerin	30–40	"
Sodium chloride	6	"
Di-Potassium phosphate	2.5	"
Ammonium lactate	6.5	"
Magnesium sulphate	0.3	"
Calcium chloride	0.1	"
Asparagin	3.2	"

Upon this medium the writer attempted to grow pure cultures of the granular, the barred, and the solid-staining[1] varieties of *B. diphtheriae*, obtained fresh from the nose and throat of patients suffering from diphtheria in the Providence hospitals. Out of 70 pure cultures tried, only two gave a growth upon Uschinsky's proteid-free medium, after an incubation of five days or more. In these two instances, furthermore, the growth was very slight and limited to the solid-staining varieties of the organism.

It is also perhaps noteworthy that in these cases, where several tubes of proteid-free media were inoculated from the *same* blood-serum culture, usually *only one or two tubes* would manifest a growth, thus seeming to indicate that even out of a pure culture of *B. diphtheriae*, there might be only a relatively small number of individual organisms which would immediately take up growth upon Uschinsky's medium; and furthermore, that these, either by chance or by peculiar fitness, proved to be the solid-staining types of the organism.

NEW COMBINATIONS.

After this trial of Uschinsky's medium, the writer made use of several other combinations of proteid-free media, all of which contained asparagin for the nitrogen basis and varied somewhat, both qualitatively and quantitatively, in respect to the salt constituents. Although an immediate growth of fresh cultures of *B. diphtheriae* upon these media did not usually occur, still on some of them the growth was more frequent and slightly more profuse than upon Uschinsky's medium. Among the many new combinations tried, especially the following may be mentioned:

Asparagin	1.50	parts
Glycerin	2.50	"
Sodium chloride	0.10	"
Di-Potassium phosphate	0.10	"
Potassium nitrate	0.03	"
Ammonium lactate	0.08	"
Distilled water to make	100.00	"

[1] Wesbrook's classification, *Trans. Assoc. Amer. Physicians*, 1900.

Upon this medium very few cultures of *B. diphtheriae* grew; in fact only two out of 20 grew at all, and these two were types of D^2 and E^2 (Wesbrook's classification).

In the following medium the percentage of glycerin and sodium chloride was increased, and calcium chloride and ammonium phosphate were added:

Asparagin	1.00 parts
Glycerin	4.00 "
Sodium chloride	0.60 "
Di-Potassium phosphate	0.03 "
Ammonium phosphate	1.75 "
Calcium chloride	0.01 "
Distilled water to make	100.00 "

In this medium six cultures grew slightly, but none very well. Of these six, three were D^2.

In the next medium ammonium lactate was omitted:

Asparagin	1.00 parts
Glycerin	4.00 "
Sodium chloride	0.60 "
Di-Potassium phosphate	0.30 "
Potassium nitrate	0.03 "
Calcium chloride	0.01 "
Ammonium phosphate	1.00 "
Distilled water to make	100.00 "

In this medium 14 cultures grew; one well, the others very slightly. Of the 14, eight were D^2, three were granular forms, and three barred. The one that grew most luxuriantly was a D^2.

Asparagin	1.00 parts
Glycerin	4.00 "
Di-Potassium phosphate	0.30 "
Magnesium sulphate	0.03 "
Potassium nitrate	0.03 "
Calcium chloride	0.01 "
Ammonium lactate	0.05 "
Ammonium phosphate	1.00 "
Distilled water to make	100.00 "

In the above medium, which, as will be noted, contains no sodium chloride, 27 cultures grew. Of these 27, seven grew fairly well, and 19 slightly. Of these 27, moreover, 12 were D^2, 6 were granular, and 9 were barred. The 2 which gave the most luxuriant growth were D^2.

The fifth lot of media had the following constitution:

Asparagin	1.00 parts
Glycerin	4.00 "
Sodium chloride	0.10 "
Di-Potassium phosphate	0.30 "
Magnesium sulphate	0.03 "
Ammonium lactate	0.50 "
Ammonium phosphate	1.00 "
Phosphate of iron	0.02 "
Distilled water to make	100.00 "

On the medium above mentioned, 18 cultures grew, most of them very slightly. Of these, 8 were D[2] or E,[2] 7 were granular, 2 were barred and 1 was a mixed culture. Owing to an accident, however, the results of growth in this series of tubes were incomplete.

In all of the above combinations of proteid-free media tried, the only source of nitrogen was either the ammonium salts or the asparagin. Generally speaking, the results in the growth or the toxin production of *B. diphtheriae* upon these media were not pronounced. In fact the growth was not sufficient to warrant the use of these media as a basis for the deeper problem of determining what definite constituents of the media favored or prevented the formation of the toxic products. But few inoculations of these first attempts at growth were made. The principal reasons for this were, that in the majority of cases the growth was too slight to even hint that there could be even the slightest toxin formation; in the second place, before the growth had progressed a suitable length of time, the rapid evaporation of the water from the tubes in the incubator so concentrated the medium that it either prevented further growth of the cultures, or produced an uncertainty as to the exact proportion of the ingredients in the solutions. It was always noticeable, however, that the forms which grew most persistently, in spite of the changes in the density of the medium, were the solid-staining varieties. Of these last, several inoculations were made, but in only one instance did the result indicate a very high degree of toxin production.

On the theory that one reason for the slight growth in the different varieties of media to which allusion has been made, might be the difficulty in assimilating the nitrogen from the ammonium compounds or from the asparagin, it was decided to make use of some other simple nitrogen-containing compounds, as glycocol or urea.

In general, the results obtained from the employment of glycocol were far more encouraging than any results obtained through the use of asparagin. Urea, however, as also determined by Sullivan[1] in his work on the biochemistry of color-production by bacteria, was of little value, either in aiding the luxuriance of growth or for increasing the formation of toxin.

The first medium in which glycocol furnished the nitrogen basis was made up as follows:

Glycerin	3.70 parts
Sodium chloride	0.60 "
Calcium chloride	0.08 "
Magnesium sulphate	0.32 "
Di-Potassium phosphate	0.23 "
Ammonium lactate	0.75 "
Glycocol	0.10 "
Distilled water to make	100.00 "

Owing to the fact that several of the first few cultures with which this medium was inoculated grew without delay, a large number of cultures was not tried. The first two cultures which grew well had been on blood serum for a period of three weeks and were transferred to the proteid-free medium from a broth culture. Of these two, one was a culture of CC^1, the other of CC^2. Of a 16-day-old culture of CC^2 in the medium specified above, 5 c.cm. were inoculated into a guinea-pig weighing 533 grams. The pig died in 36 to 38 hours with the characteristic signs of diphtherial poisoning. The organism was recovered at the autopsy in the form of a D^2 culture.

In still another case, a culture of CC^2, having been grown for a period of 17 days in media of the same composition as the foregoing, killed a pig in 31 hours with typical diphtherial poisoning. In this case also, the organism was recovered in the form of a culture of C^2D^2. These results appear to indicate that glycocol, even in so small an amount as 0.1 per cent, is able to render possible, provided the other constituents of the medium are favorable, a good growth and a powerful toxin production of *B. diphtheriae* upon proteid-free media, even if the period of growth is no longer than 16 days. We recall that Uschinsky states that the period of growth of his cultures was from four to six weeks.

[1] Sullivan, *Jour. Med. Res.*, 1905, 14, p. 109.

Another modification of the medium was made by substituting urea for the glycocol as the nitrogen basis.

Glycerin	15.00 parts
Sodium chloride	3.00 "
Calcium chloride	0.05 "
Magnesium sulphate	0.15 "
Di-Potassium phosphate	1.25 "
Ammonium lactate	3.20 "
Urea	1.60 "
Distilled water to make	500.00 "

In the above instance the growth was even slighter than when asparagin was used; and this was true for all the tubes inoculated. Consequently no inoculations from these cultures were made.

A further modification was attempted by combining urea and glycocol in the same medium, as follows:

Glycerin	17.00 parts
Sodium chloride	3.00 "
Calcium chloride	0.04 "
Magnesium sulphate	0.16 "
Di-Potassium phosphate	1.25 "
Ammonium lactate	3.25 "
Glycocol	0.50 "
Urea	2.50 "
Distilled water to make	500.00 "

A 17-day-old culture of CC^1 upon this medium was inoculated into a guinea-pig and death resulted in 59 hours. In this case, at least, urea appeared to hinder rather than to favor either the luxuriance of growth or the degree of toxin production; for the same form of *B. diphtheriae*, grown for the same time and in the same medium, but without the urea, killed in from 36 to 38 hours.

TABLE 1.

COMPOSITION OF SOME OF THE MEDIA USED IN THE EXPERIMENTS.

	1			2			3			4		
Am. Lactate	.65			0.08						0.05		
Am. Phosphate							1.00			1.0		
Asparagin	.32			1.5			1.0			1.0		
Cal. chloride	.01						0.01			0.01		
Ferric phosphate												
Glycerin	3 to 4			2.5			4.0			4.0		
Glycocol												
Magnesium sulphate	.03									0.03		
Potas. nitrate				0.03			0.03			0.03		
Di-Potas. phosphate	.25			0.1			0.3			0.3		
Sodium chloride	0.6			0.1			0.6					
Urea												
	CD	C^1D^1	C^2D^2	CD	C^1D^1	C^2D^2	CD	C^1D^1	C^2D^2	CD	C^1D^1	C^2D^2
Growth		S	S			S	S	S	G	S	S	G
Toxin production		O·	O									

TABLE 1.—*Continued.*

	5	6	7	8
Am. Lactate	0.5	0.75	3.2	3.25
Am. Phosphate	1.0			
Asparagin	1.0			
Cal. chloride			0.05	0.04
Ferric phosphate	0.02	0.08		
Glycerin	4.0	3.4	15.0	17.0
Glycocol		0.1		0.5
Magnesium sulphate	0.03	0.32	0.15	0.16
Potas. nitrate				
Di-Potas. phosphate	0.3	0.23	1.25	1.25
Sodium chloride	0.1	0.6	3.0	3.0
Urea			1.6	2.5

	CD	C^1D^1	C^2D^2	CD	C^1D^1	C^2D^2	CD	C^1D^1	C^2D^2	CD	C^1D^1	C^2D^2
Growth	S	S	S	L	L	L		S	L	S	S	L
Toxin production				H	H					M	M	

EXPLANATION.—In every case except Nos. 7 and 8 water was added to make 100 parts; in these to make 500 parts. S=slight, G=good, L=luxuriant growth; H=Inoculation resulted in death in less than forty-eight hours; M=Inoculation resulted in death in more than forty-eight hours.

It may be stated here that the three last-mentioned combinations of proteid-free medium were also tried, made up with agar in solid form. Although many other forms of bacteria developed luxuriantly upon these media, the growth of *B. diphtheriae* was slight. It was, however, in several instances sufficiently rapid to enable the writer to make a diagnosis of the diphtheria organisms after a period of 12 hours' growth.

STUDY OF ADAPTATION.

One other point of incidental interest was to determine the possibility of adapting to a proteid-free medium cultures of *B. diphtheriae* which, when fresh from the throat of man, would not grow at all on the proteid-free combinations. It is a well-known fact that many plants may, through a long and gradual process of adaptation, be fitted to live and grow in a new and materially different environment. In the present case the adaptation process was carried on as follows: To one part of broth were added portions of the proteid-free medium, in the following parts: 1, 2, 4, 6, 8, 10, 14, 18, 25, 30, 40, 50, 60, 80, 100, 140, 180, 210, 240, until finally the proteid-free medium was reached in its purity. In this system of adaptation, the tubes were inoculated successively, each from the tube preceding. A period of from 24 to 36 hours was allowed for the growth in each of the first tubes of the series, while for the tubes in the last of the series a longer period of time was found to be necessary.

By this process of adaptation five cultures of *B. diphtheriae*, of which two were of the D^2 type and the other three the CC^1 type, were brought successfully to the eighteenth series of adaptation tubes, in which the medium contained one part of broth to 210 parts of proteid-free medium. In every instance the solid-staining forms grew the most luxuriantly. In every instance, moreover, except in one culture of D^2, a strong toxin was developed, and the degree of toxicity appeared to be directly proportional to the age of the culture. After the cultures had once taken a hold, so to speak, any increment in the proteid-free basis appeared to make little difference in the strength of the toxin produced in the tubes beyond the fourth series (i. e., broth 1 part, proteid-free medium 6 parts). The records of the inoculations of the barred and the granular forms during the process of adaptation may be tabulated as follows:

TABLE 2.

Series	Age of Culture	Composition	Killed in
1.....	3 weeks	Broth 1 pt.; Prot.-free 6 pts.	25 hours
2.....	2 weeks	Broth 1 pt.; Prot.-free 80 pts.	32 hours
3.....	3 weeks	Broth 1 pt.; Prot.-free 210 pts.	28 hours

These results seemed sufficiently definite to warrant the statement that certain forms of the diphtheria organism which will not grow normally upon a proteid-free medium can, by slow degrees, be fitted to such a life, and may be made to produce under such new life conditions a very strong toxin. There still remains a problem of interest to be solved in determining whether the adaptation results from a modification of the existing forms, or whether a selection of those varieties which are most suited to the new environment takes place. Regarding this question there are no data to be brought forward at this time.

PERIOD NECESSARY TO OBTAIN MAXIMUM TOXICITY.

There is a general unity in the opinion that the maximum toxicity for *B. diphtheriae* growing in broth is reached in from 40 to 50 hours after the inoculation. Uschinsky, on the other hand, ascertained that a period of from four to six weeks was required for the formation of a powerful toxin in his proteid-free media. In the tests which the

writer made in this regard, it was quite apparent that the food materials in a proteid-free medium were much more slowly assimilated than in the case of a broth culture. In the majority of cases at least a day was necessary before the growth was at all noticeable; and the growth was seldom heavy before three to five days had elapsed. A number of instances appeared to indicate that the degree of toxicity, at least within certain limits, was directly proportional to the time that the culture had been in incubation.

RELATION OF THE DEGREE OF TOXICITY TO THE FORM OF THE ORGANISM AND TO THE LUXURIANCE OF GROWTH.

There can be but little doubt that the granular forms, so called, of *B. diphtheriae* are, as a rule, the most virulent. The solid-staining varieties, on the other hand, appear to be non-pathogenic in the greater number of cases. And yet the results of the writer's observations permit of little doubt that a single morphological variety of the diphtheria organism is decidedly modifiable; and that not only may the granular types of the organism be resolved into the solid-staining forms, but that the opposite may also be true. Furthermore, it is clear that the solid-staining types may, either with a maintenance of their original form, or with a change of that form within the body of the animal, prove highly pathogenic for guinea-pigs. The observations in this regard, were only incidental to the main problem in hand; but, in view of the prevalent difference of opinion upon this question, they may perhaps be appropriately mentioned at this time. On the first trial, eleven inoculations of pure cultures of D^2 were given to guinea-pigs before one culture proved to be pathogenic. The twelfth, a D^2 type, killed in 39 hours, with all the usual signs of diphtherial poisoning. When the organism was again recovered, it was found to be of the barred variety. On several other occasions also, when granular forms were inoculated, barred and solid-staining forms were recovered. In three successive cases where two cultures of CC^1 and one culture of $C^1 C^2$, grown for 17 days upon proteid-free media, were inoculated into guinea-pigs, they killed in from 37 to 59 hours, with all the characteristic symptoms. Without an exception, when the organism was recovered from the bodies, the D^2 form was alone present. One of these recovered cultures of D^2, after 48 hours of incubation in broth, was inoculated into a guinea-pig and resulted in

death in 37 hours. The organism was recovered in the form of a D^2.

These facts appear to indicate beyond a doubt that important changes may be brought about in the form and toxin production of individual bacilli in a single culture of the organism; and that these different forms are determined in a great measure by the nature of the environment. Whether this distinct modification occurs as a result of a morphological change in all the individuals during a few successive generations, or whether by a less rapid process of selection and elimination of those morphological varieties, or different strains of the same morphological variety, which are not readily adaptable, this we are not yet able to determine. It is not altogether improbable, however, that there may be in the life of the diphtheria organism what may be roughly called a series of adaptive forms, each one of which may be best suited to a circumscribed condition of environment, there to produce, as the case may be, color, toxin, or even definite changes in form.

TOXICITY AND LUXURIANCE OF GROWTH.

As to the relation between the degree of toxicity and the luxuriance of growth, the experience of the writer would not warrant the drawing of any far-reaching conclusions. It is true, however, that the solid-staining types, whether they be true *B. diphtheriae*, pathogenic pseudo-diphtheria bacilli, or non-pathogenic pseudo-diphtheria bacilli grow more rapidly upon proteid-free media than do the granular types. Not only do the D^2 forms grow more rapidly, but the nature of their growth is far more luxuriant and of quite a different nature from that of the granular varieties. The typical growth for the granular type, on either broth or proteid-free media, is at the surface, where it forms a more or less delicate film. Upon shaking the tube, this film falls to the bottom as a fine precipitate or sediment. The medium from the surface to the bottom of the tube is, in the growth of the granular forms, nearly as clear as the contents of a sterile tube. It is the nature of the solid-staining types, on the other hand, instead of forming the surface growth and maintaining a clear mid-liquid, to produce in the medium a uniform cloudiness. This may, in time, settle to the bottom of the tube, but the medium never wholly loses its turbidity. This condition of growth was observable in all the

series of cultures in proteid-free media as well as in the latter part of the series of adaptation tubes which have been mentioned above. In summation it may be said that, although a high degree of toxicity may often accompany a luxuriant growth, luxuriance of growth can never be used as a criterion of the degree of toxicity.

It may be of interest to mention one other point regarding the so-called involution-forms of *B. diphtheriae*. These, known by Wesbrook's classification as the "A" varieties, are generally conceded to represent forms which have become attenuated, both in virulence and in luxuriance of growth. This is indicated by the fact that when a culture of *B. diphtheriae* is grown upon a medium unfavorable to its growth, many of these forms develop, and they are often common in the throats of those who are recovering from an attack of diphtheria. In the writer's experience, these forms often developed, at the very last, in tubes of proteid-free media wherein the granular, and less frequently the solid-staining types, had found growth most difficult. These same involution-forms, in at least three cases, in our experiments, produced such a rapid and luxuriant growth in broth, that we were deceived by their macroscopic appearance into believing that these particular tubes were contaminated with some one of the large, pellicle-forming spore-bearers. By the examination of one tube, however, it was found that there was naught in the tube but a pure culture of the "A" varieties of *B. diphtheriae*, and that these composed, not only the heavy pellicle, which, in 24 hours, had become so dense that it restrained the liquid portion when the tube was inverted, but also the rapidly growing individuals beneath the pellicle. These individuals were the largest that have ever come under our observation, many of them measuring nearly ten micra in length, and under $\frac{1}{16}$-inch oil-immersion, rendering camera lucida drawings easily possible. It may also be noted that two of these cultures, referred to above, came originally from monstrous colonies growing upon blood-serum, one of which was 10 mm. in diameter. These were the only colonies of this nature ever seen by the writer.

SUMMARY.

1. *B. diphtheriae* will grow readily and luxuriantly in proteid-free media, and will produce therein as strong a toxin as in ordinary broth, though much more slowly.

2. Of the three nitrogen bases tried (asparagin, urea, and glycocol), urea seemed to be of slight value, while glycocol furnished the best growth and the strongest toxin. Asparagin appeared to give better results than urea, though it was not as satisfactory as was glycocol. There is no added value to be gained by using any two, or even three, of these compounds together.

3. Upon the following medium, cultures of *B. diphtheriae* were grown, and, after an incubation of 16 days, killed guinea-pigs in the course of 36 to 38 hours:

Glycerin	3.40 parts
Sodium chloride	0.60 "
Calcium chloride	0.08 "
Magnesium sulphate	0.32 "
Di-Potassium phosphate	0.23 "
Ammonium lactate	0.75 "
Glycocol	0.10 "
Distilled water to make	100.00 "

4. In several instances there were obtained cultures of pathogenic, solid-staining varieties of *B. diphtheriae* (C^2, D^2, E^2), which, upon inoculation, killed guinea-pigs in 37 to 60 hours.

5. Cultures of *B. diphtheriae* which will not at first grow upon proteid-free media, may be adapted to it by slow degrees; and whether during the process of adaptation, or after the adaptation, it is the solid-staining forms of the diphtheria organism which always manifest the most rapid and luxuriant growth.

6. A single morphological variety of *B. diphtheriae* is decidedly modifiable, and there are indications that there may be in the life of the diphtheria organism what may be roughly called a series of adaptive forms, each one of which is best suited to a certain condition of environment, where it may produce color, toxin, or change in form.

THE LIBERATION OF FORMALDEHYDE THROUGH THE AGENCY OF CALCIUM CARBIDE.

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DURING the last two years a very considerable amount of work has been done at the Maine Laboratory of Hygiene upon the matter of formaldehyde disinfection. In the course of this work the formalin-permanganate method was first worked out as a means of practical disinfection. In one way this method seemed an anomaly, in that part of the agent which was to do the work of disinfection was itself used up in the reaction that furnished the heat to evaporate the formaldehyde solution. In looking for a means of surmounting this difficulty, the use of calcium carbide was suggested, and the subsequent work with this compound forms the basis of this paper.

As is well known, when calcium carbide and water are mixed, there results a violent reaction from which acetylene gas is formed and driven off, while calcium oxide is theoretically the product remaining in the generator. In actual practice the residue in the generator is calcium hydroxide, resulting from the union of the first-formed oxide with water. The value of this reaction for disinfection does not lie in the products formed, but in the heat which results from the reaction. It was purposed to use this heat to evaporate the formaldehyde that was to be added to the calcium carbide, just as in the formalin-permanganate method the heat resulting from the oxidation of a portion of the formaldehyde was employed to evaporate the unoxidized portion. In this case it seemed probable that there would not be the destruction of formaldehyde noted in the other method.

In the work ordinary commercial calcium carbide and formaldehyde solution were used. The particular lot of formaldehyde solution used assayed 35.27 per cent by weight of formaldehyde. These reagents were used, as it was desired to get only such results as would be obtained in actual disinfection by this method, if it turned out to be of any value.

The first work necessary was to determine the proportions of the reagents to be used. In this work it was first noted that when the

formaldehyde was added to the calcium carbide, the formaldehyde being in its original strength, there was practically no reaction. In other words there did not seem to be enough water to react with carbide in the formalin solution alone. By using enough carbide to leave a thoroughly dry residue in the generator it was found that the formalin solution should be diluted by its own volume of water in order to give the best results. Starting then with a solution half water and half formalin, a large number of tests were made to determine the amount of carbide necessary to give a dry residue in the generator and at the same time leaving as little as possible of the carbide unused. It was finally decided to use six grams of the carbide to every 10 c.c. of the diluted formaldehyde solution. Experiments in using the large amounts of the reagents in the disinfecting-room gave as satisfactory results with these proportions as did the smaller amounts in preliminary experiments.

The work done in determining the proper proportions of the reagents showed that the best results were to be obtained when the carbide was in size about that of a pea. If in a powdered form the reaction was too violent, and if much larger than a pea the reaction was so slow as to produce an insufficient amount of heat to evaporate the formaldehyde. At the same time it was noted that a rather low dish, with walls about eight or ten inches high, and with a very wide bottom was preferable for a generator. The reagents and generator being as above described, the reaction, which starts rather slowly, is over in between six and seven minutes.

To determine the amount of formaldehyde liberated by this reaction, air was drawn from the room in which disinfection was going on. The apparatus used for this purpose was the one formerly used in determining the formaldehyde in rooms charged by the formalin-permanganate method, fully described in the paper on that work in the *Fourteenth Report of the Maine Board of Health.* In brief the apparatus was this:

A glass tube was passed through a hole in the door of the disinfecting-room so as to project about three feet into the room, the end in the disinfecting-room terminating in a funnel. The end of this glass tube, which was outside the disinfecting-room, was connected to the first of a series of three Dreschsel gas bottles, each containing 75 c.c. of distilled water. The last of the series of Dreschsel bottles was connected with a 10-liter bottle, filled to the 10-liter mark with water, any amount of which

could be siphoned off at pleasure. All rubber connections were sealed with paraffin wax and thoroughly tested to see that they were air-tight. The stoppers of the several bottles were also sealed in with paraffin wax.

In practice 20 minutes were allowed to elapse after the reaction was over before the siphon was started, so that there might be time for the gas to diffuse uniformly throughout the room. The apparatus was then allowed to run at such a rate that at least 35 minutes were required to draw 10 liters of air through the Dreschsel bottles. The pressure conditions were then equalized and the gas bottles were disconnected, their contents were poured into a 500 c.c. flask, each bottle washed three times with distilled water, and the washings added to the contents of the 500 c.c. flask. To the contents of the flask were now added 10 c.c. of a solution of standard potassium cyanide; the flask was well shaken, allowed to stand for five minutes, then 10 c.c of a solution of N-10 silver nitrate, previously acidified with 10 drops of 75 per cent c.p. nitric acid, added; the whole made up to 500 c.c.; mixed; 100 c.c. filtered, and the excess of silver in the filtrate determined by Volhard's method. From this the amount of formaldehyde in the 10 liters of air examined was calculated, and, knowing the cubic contents of the room and the amount of formaldehyde introduced, the percentage of the original amount of formaldehyde that was yielded by the reaction was easily calculated. On three different tests the amount of formaldehyde found in the room ranged from 6.12 per cent to 11.4 per cent.

When the room was opened at the end of four hours the odor of formaldehyde was not so strong as to cause any annoyance to either the eyes or to the throat, even when the formaldehyde was used in the proportions of 500 c.c. to 1,000 cubic feet. In fact there was not so much trouble to be experienced in entering the room thus charged as there was in entering the same room when charged by the formalin-permanganate method with 200 c.c. to 1,000 cubic feet. Neither was the odor of acetylene very strong. It cannot be expected that all of this loss of formaldehyde is to be accounted for by leakage, as this did not happen when simply formaldehyde was evaporated into the room. It is possible that the mixture of the two gases—acetylene and formaldehyde—may diffuse more rapidly than formaldehyde alone, as a stronger odor of acetylene was always to be noted outside of the disinfecting-room than was ever the odor of formaldehyde, when this alone was used in charging.

Just before the completion of the reaction, when the effervescence was at its height, a yellowish-brown scum began to form over the entire surface of the liquid, and this rapidly thickened, so that in a short time the gas seemed to have difficulty in forcing its way through the scum. This scum seemed to increase in amount from the time of its appearance until the end of the reaction. No chemical examination was made of this scum, as the object of the work was to deter-

mine the bacterial efficiency of the method rather than to determine the chemical products formed. It is possible that the aldehyde, in the presence of the hot alkali, formed some solid condensation product, as aldehydes often do under these conditions. This would of course cause a considerable loss of formaldehyde for disinfecting purposes. At any rate the yield of formaldehyde by this process is very low, and the bacterial results reflect this condition.

The bacterial work was done in a room having a capacity of 862 cubic feet, measuring 20×4 ½×10 feet high, and containing a small jog at one end. The room had one door measuring 3×7 feet, and two windows. One was on the outside of the building, measuring 3 ¼×6 ½ feet, and the other opened into another room. This window was 8 feet from the floor, and measured 1×9 ½ feet. No attempts were made to make the door or windows air-tight, they being left in their natural condition so as to have disinfecting conditions as near those actually encountered by the health officer. The interior of this room was papered. The technique of the bacterial work was as follows:

A piece of heavy glazed paper was bent so as to form a shelf. Then five strips of filter paper were inoculated by loops of broth or heavy smears of blood-serum cultures of the organisms to be used. Each strip was picked up with sterile forceps, and, with sterile shears, cut into two pieces. One piece was put on the shelf of glazed paper, and the other dropped into a tube of bouillon as a control. Five strips, inoculated with the same organism, were put on each paper shelf, and the latter then placed in the disinfecting-room. Here the paper shelves were distributed in all parts of the room, and also at all heights from the floor to the ceiling. When the requisite number of cultures were in position the reagents were mixed and the room closed for four hours. At the end of this time each paper shelf was removed from the room; the pieces of infected filter-paper picked up with sterile forceps; introduced into separate tubes of sterile bouillon, and incubated at 37.5° C. for 196 hours, at the end of which time they were examined. All growths were examined microscopically to see if they were those put upon the paper or were the result of contamination of some sort.

In the work with the formalin-permanganate method the proportions of 200 c.c. to 1,000 cubic feet gave very satisfactory results and work was started using these proportions. The cultures used were colon, diphtheria, pneumococci, pyocyaneus, albus, tetragenus, typhoid, prodigiosus, and mixed cultures from throat swabbings. The whole number of cultures exposed here was 335. The work was done on three separate days, the temperatures being respectively 73°, 74°, 74° F. The average increase in the humidity in the test-room was 11.3 per cent.

TABLE 1.

DISINFECTION TEST, 200 C.C. TO 1,000 CUBIC FEET.

Culture	Number	Growth	No Growth
Colon	35	1	34
Diphtheria	30	7	23
Pneumococci	35	4	31
Pyocyaneus	40	20	20
Albus	45	13	32
Tetragenus	30	2	28
Typhoid	25	2	23
Prodigiosus	30	9	21
"Mixed"	65	20	45
Total	335	78	257

These proportions not giving satisfactory results the amount of formaldehyde was raised to 300 c.c. to 1,000 cubic feet. Here again work was spread over three separate days, their respective temperatures being 79°, 81°, and 70° F., and the average rise in humidity in the test-room was 11.8 per cent. Three hundred and forty-nine cultures were exposed; 168 of these were buried cultures. The results follow.

TABLE 2.

DISINFECTION TEST, 200 C.C. TO 1,000 CUBIC FEET.

Culture	Number	Growth	No Growth
Pneumococci	25	2	23
Pyocyaneus	48	4	44
Albus	53	0	53
Streptococci	9	0	9
Typhoid	48	4	44
Prodigiosus	23	2	21
Colon	25	1	24
Tetragenus	16	2	14
Diphtheria	31	19	12
Aureus	20	19	1
Throat	26	2	24
"Mixed"	25	19	6
Total	349	74	275

The results being again unsatisfactory the proportions were raised to 500 c.c. to 1,000 cubic feet. The work was done on three separate days, the temperatures being 74°, 79°, and 76° F. The average increase in the humidity was 13.2 per cent. In all, 268 cultures were exposed of which 120 were buried and 148 were open cultures. The method of burying the cultures was that used in my work on the formalin-permanganate method, and there fully described. In brief it was as follows:

A piece of tin six inches square had a circular hole cut in the center. Over this were spread the requisite number of thicknesses of whatever kind of cloth was used—

silk, cotton-flannel, or ticking. On the upper layer of the cloth were placed five inoculated slips of filter-paper, so as to be over the circular hole. On top of these was spread the same number of cloths as were below, the whole being capped by duplicate of the tin bottom, and then the edges were clamped tight so that the only way for the gas to reach the organisms was by penetrating the layers of cloth exposed by the holes in the tin.

TABLE 3.

DISINFECTION TEST, 500 C.C. TO 1,000 CUBIC FEET.

Culture	Number	Growth	No Growth
Colon	30	0	30
Diphtheria	40	0	40
Pneumococci	30	0	30
Pyocyaneus	20	0	20
Albus	13	0	13
Typhoid	63	0	63
Subtilis	22	5	17
Anthrax	30	0	30
"Mixed"	20	0	20
Total	268	5	263

From these results it seems that it is possible to get efficient disinfection by this method provided that use is made of not less than 500 c.c. of formaldehyde solution for every 1,000 cubic feet of room space to be disinfected. The method does not compare favorably with the formalin-permanganate method, it requiring twice the amount of formaldehyde for efficient disinfection that the latter does. With the proportions used by me there was not such an increase in the humidity of the test-room as there was with the permanganate method. It may be that under some conditions this method may be of use. The acetylene and formaldehyde gases had no more effect on flies and mosquitoes than does formaldehyde gas alone.

TABLE 4.

SUMMARY

	Growth	No Growth	Total
500 c.c.	5	263	268
300 c.c.	74	275	349
200 c.c.	78	257	335
Total	157	795	952

THE LIBERATION OF FORMALDEHYDE GAS FROM SOLUTION BY MEANS OF POTASSIUM PERMANGANATE.

G. B. FRANKFORTER.

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SEVERAL years ago, the writer, while studying the action of formaldehyde on camphoroxime, accidentally brought some methyl alcoholic solution of formaldehyde in contact with powdered potassium permanganate. The result was a violent explosion. A short time later the experiment was repeated, using methyl and ethyl alcoholic solutions of formaldehyde and also with the common 40 per cent aqueus solution. The results were not at all as expected. Instead of a violent explosion, a vigorous reaction took place, producing a large amount of heat and liberating formaldehyde gas.

At the time these experiments were made, formaldehyde was not used extensively for disinfecting purposes, so that the chief interest in the reaction centered about the amount of heat produced. A great number of calorimetric determinations were made. The results, however, were not entirely satisfactory. Variable results were obtained, although experiments were exactly duplicated. Early in these calorimetric determinations it was observed that when the formaldehyde was slowly dropped on powdered potassium permanganate, as each drop came in contact with the permanganate a little tongue of fire was produced and the amount of carbon dioxide was increased. These calorimetric determinations were suddenly stopped, owing to the fact that a second explosion occurred, badly injuring the calorimeter.[1]

In the study of the heat of combustion, a number of difficult problems had to be solved before satisfactory calculations could be made. First, the amount of formaldehyde liberated had to be determined as well as the polymerized formaldehyde left in the permanganate residue;

[1] A third explosion occurred some years later, while Mr. Rodney West was continuing these heat determinations. In this case the force was so violent as to render him unconscious, and these heat determinations were again discontinued.

and second, the amount of carbondioxide and formic acid had to be determined. In the determination of formaldehyde, the various methods were tried and were not found satisfactory, especially in the determination of the paraformaldehyde left in the residue. A new method was therefore worked out whereby the aldehyde in either form could be determined accurately and without great difficulty.[1] The above method depends upon the fact that when formaldehyde is brought in contact with hydrogen peroxide in the presence of an alkali, pure hydrogen is liberated according to the following reaction:

$$2CH_2O+H_2O_2+2KOH=2HCOOK+2H_2O+H_2.$$

This method was carefully compared with the other methods and used in all the determinations which follow. It is believed that this method for determining formaldehyde will supplant the titration methods, as nothing but potassium hydroxide and hydrogen peroxide and a graduated tube are necessary for the determination.

In these determinations of formaldehyde gas liberated by potassium permanganate, it was found that the conditions under which the substances are brought together would vary the amount of formaldehyde gas. The condition of the permanganate, whether in crystal form or whether coarsely or finely powdered, made a great difference in the reaction. The strength of the formaldehyde solution and the rate of flow upon the permanganate likewise make a great difference both in the amount of formaldehyde gas liberated and in the amount of heat produced. In fact, so many variables enter into the reaction, that the calorimetric determinations were laid aside for a time and the conditions under which the greatest amount of formaldehyde gas could be liberated by permanganate were studied.

DETERMINATION OF FORMALDEHYDE GAS.

Several obstacles were encountered in the determination of the amount of formaldehyde gas liberated from its solution by potassium permanganate. One of the first difficulties was the retention of the gas, so that it could be accurately determined, the evolution of gas being so violent that the ordinary gas-bag stop-cock is not sufficiently large to allow the gas to escape without producing an enormous pressure upon the generator. Again, in the case of absorption of the gas,

[1] See "Gasometric Determination of Formaldehyde," *Jour. Amer. Chem. Soc.*, 27, p. 714.

it is extremely difficult to retain all of the gas either by water or by ammonia.

Another difficulty was experienced in bringing the formaldehyde solution in contact with the permanganate. If aldehyde is allowed to drop on permanganate from a burette, the permanganate which comes in contact with the aldehyde is changed to the oxides of manganese which seem almost impervious to the aldehyde solution, thus preventing further action. If, on the other hand, the formaldehyde is added all at once, the gas is liberated almost with explosive violence, but the amount of gas liberated is considerably less than when the solution is is poured slowly over the permanganate.

An attempt to overcome this violent reaction was made by diluting the formaldehyde solution with water. It was found, however, that while the addition of water modified the reaction, the quantity of formaldehyde gas liberated was greatly diminished. Finally, the dilution of the permanganate by some inert substance was tried and found to give satisfactory results. Potassium permanganate was powdered and mixed with clean sand and the formaldehyde slowly added to this mixture. The sand prevented the formation of the impervious coating of oxides of manganese. The solution percolated through the mixture and the reaction continued as long as the aldehyde was added, or as long as there was unchanged permanganate left in the generator. As the gas is liberated from the solution by the heat of the reaction and as the specific heat of the sand is much less than that of water, more heat and hence more gas must necessarily be liberated by diluting the permanganate with sand than by diluting the formaldehyde solution with water. After many trials, it was found that a mixture of equal parts of sand and permanganate gave best results. With this proportion the reaction was violent, but not to such an extent as to make the collection of the gas impossible.

Several forms of apparatus were tried. That indicated in Fig. 1 was adopted as giving satisfaction, whether the gas was collected in gas bags and measured, or determined by absorption.

A wide-mouth, short-neck flask *A*, of about 200 c.c. was fitted with a double-bored rubber stopper. Through one hole passed a burette for introducing a known quantity of formaldehyde. Through the other passed a delivery tube *E* of large diameter. This delivery tube was connected with an aspirator bottle *B*, filled with water, one liter giving best results. The side neck of the aspirator bottle was con-

nected with an absorption tube *F*. The absorption tube was so placed that the upper end was below the surface of the water in *B*, thus slightly diminishing the atmospheric pressure upon the gas. The other end of the absorption tube was connected with another aspirator bottle *C*, which serves as a receiver in case the gas is not entirely absorbed as soon as it passes into *B*. If the reaction is very violent, forcing all of the water out of *B* into *C* by rapid liberation of gas, loss will be prevented by bubbling through the water in the absorption tube. At the end of the reaction the water is finally drawn back into *B*, but is prevented from being drawn over into *A* by the air which filled the generator at the beginning of the experiment. Usually, the absorption tube is unnecessary except as a connecting tube between *B* and *C*. When all the gaseous formaldehyde has been absorbed by the water, it is thoroughly mixed, measured, and the formaldehyde determined. During the reaction the temperature in the generator is practically constant at the boiling-point of water.

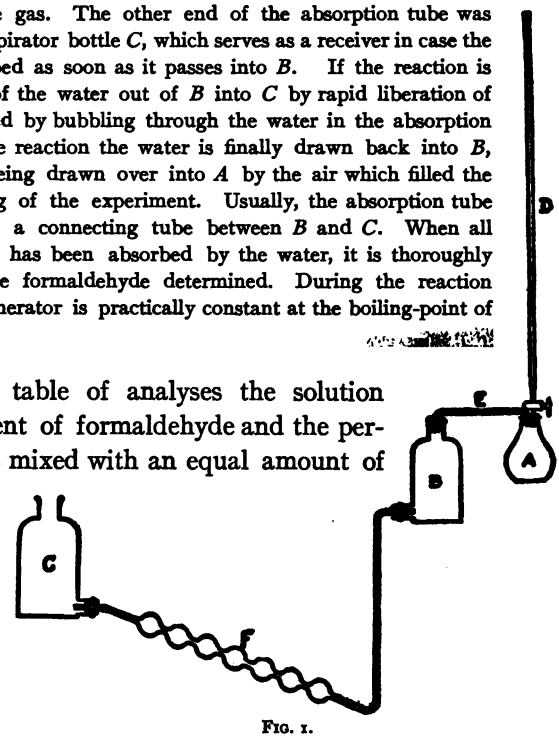

FIG. 1.

In the following table of analyses the solution used was 37.8 per cent of formaldehyde and the permanganate used was mixed with an equal amount of sand. The quantity of water used in absorbing the gas was varied, but 500 to 1,000 c.c. seemed to give the best results when 50 c.c. of the formaldehyde solution were used. With very large quantities of water the solution became so dilute as to make an exact determination of formaldehyde somewhat difficult.

TABLE 1.

	$KMnO_4$+Sand Grams	Formaldehyde Solution.	Volume of Water Used in Absorption	CH_2O Liberated Per Cent
1.....	100	50 c.c.	1,000 c.c.	69.12
2.....	100	50	1,000	71.42
3.....	150	50	1,000	65.92
4.....	150	50	1,000	63.53
5.....	200	50	1,000	71.77
6.....	200	50	1,000	70.00
7.....	200	50	500	64.53
8.....	200	50	500	64.53

A large number of determinations were made, varying the time of reaction or the rate of flow of formaldehyde solution on the perman-

ganate. The following table gives the average percentage of gas liberated:

TABLE 2.

Number of Analyses	Time of Reaction	CH_2O
	Minutes	Per Cent
8	80	65.96
3	60	68.06
6	45	73.11
8	30	74.56
2	15	73.56
9	10	70.48

In these determinations 200 grams of the mixture of sand and permanganate and 50 c.c. of 38 per cent formaldehyde were used. During the process a considerable quantity of water distilled over. The greater part of the water came from the formaldehyde solution; a small quantity was formed by the oxidation of the formaldehyde. Measurements showed that about 45 c.c. distilled.

The above averages indicate that, with the quantities of permanganate and formaldehyde used in these experiments, the best results were obtained by allowing 30 minutes to complete the reaction. When more than that is taken, the amount of formaldehyde liberated seems to decrease and the amount of carbon dioxide to increase. With less than 30 minutes to complete the reaction, the amount of formaldehyde decreases and the amount of carbon dioxide seems also to decrease. Determinations of both carbon dioxide and formic acid are now being made. Most of this work was done with Mr. Rodney M. West, now chemist in the Minnesota State Food and Dairy Commission.

1. SPUTUM SHAKING AND SEDIMENTING APPARATUS. 2. A RABIES COLLECTING OUTFIT.

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I. APPARATUS FOR SHAKING AND SEDIMENTING SPUTUM SPECIMENS.

A. The shaking of sputum.—Anyone who has had occasion to make many examinations of sputum for tubercle bacilli appreciates the difficulty in uniformly securing a good even smear, thick enough to present a fair sample of the particular specimen under examination, yet not so thick as to obscure the light or jeopardize the chances of finding the organism if present. If the specimen be chiefly mucous in character the difficulty in obtaining caseous particles imbedded therein is greatly increased, and often it is only by the exercise of the greatest patience and care in manipulation that satisfactory results are obtained. One often has an uncomfortable feeling, in cases where the clinical signs are fairly well marked but where bacteriological results are negative, that the organisms are probably present in the lungs, but are being raised in only very small numbers and have escaped observation.

To overcome the difficulty in smearing and at the same time to increase the efficiency of the examination of sputum, it occurred to the writer to investigate the value of digesting, shaking, and sedimenting the specimens.

It was at once apparent that unless the entire process was carried out in the small one-ounce bottles supplied by the laboratory, manipulations would become tedious and confusion of samples might take place. Moreover to secure a complete breaking up of all particles and a resulting homogeneous mixture a rapid short stroke was necessary. A vertical position for the bottle and for the direction of the stroke had the advantage of being convenient and at the same time gave more distance for the contents of the bottle to travel than a simple horizontal motion. Since all shaking machines on the market gave either a long stroke, worked horizontally, or were unavailable for the purpose in hand, the following apparatus was devised:

On an iron platform 14 x 10 inches is mounted a steel shaft running from the center lengthwise to one end, where a heavy flywheel is hung, the platform being raised from the floor by short iron legs. In the center of the platform a truncated metal cone is bolted the two connecting surfaces having a ground joint.

On the top of the truncated cone a steel cylinder is mounted by means of a threaded joint, the cylinder being capped by a steel disc riveted to the sides. The center of this disc or plate is cut out to receive a piston-rod of about the same length as the cylinder. The piston-rod is attached at its lower end to a connecting rod, which in turn is joined to the shaft. The lower joint runs in oil, while the upper joint is splashed with oil when the machine is running. About midway of the piston rod a plate is attached which fits the cylinder closely and which supports a flat steel spring. As the piston rises, air is compressed between this plate and the cap of the cylinder and escapes through several small valve holes in the sides, this together with the spring reducing the jolt of the upward stroke to a minimum.

A second-steel cylinder, to which is attached a broad platform carrying small metal cups, slides upon the inner cylinder. The top of the outer cylinder is capped and the cap is bolted to the top of the piston rod, an up-and-down motion thus being conveyed to the outer cylinder and the platform. As the outer cylinder rises, an air space is formed between the cap of the outer and that of the lower cylinder. The jolt of the downward stroke is thus absorbed in the same manner as that previously described for the upward stroke. The machine can be either directly connected with a small motor, or belt driven. A speed of 500 strokes per minute for from five to ten minutes has been found satisfactory.

At one side of the platform a short lever is provided to drive the stoppers of the sputum bottles home, the same level being obtained with each one by means of a small stop fitted with a milled thumbscrew. The bottles fit snugly in the metal cups provided on the platform, and are held in place by a heavy metal cap-plate which fits over the top of the outer cylinder and which is fastened down by two small thumbscrews.

By means of this apparatus all masses coagulated by the 5 per cent carbolic acid sent out in the bottles and all caseous particles in the sputum are rapidly broken up and the sputum rendered homogeneous throughout. The time necessary to smear a dozen specimens properly is materially reduced, and the smears are more even and less apt to flake.

A sufficient number of comparative tests has been made to show that the total number of positives is increased somewhat by this method.

Out of 1,000 specimens examined 212 were positive both before and after shaking. Four samples gave positive results before shaking and negative results after, while 10 gave negative results before and positive results after shaking.

In other words there is a loss of about 1.7 per cent in positive

results by shaking, but also a gain of about 4.5 per cent, giving a total gain of about 2.8 per cent.

The only explanation for the decrease after shaking that the writer has to offer is that possibly if only a very few bacilli are present, say, for instance, in a single caseous particle, this might be fished for and obtained before shaking, but the organisms after shaking might be so scattered as to be missed entirely.

Note was made in every case where there was a comparative increase or decrease in the number of bacilli present. In 29 cases there was an increase and in 10 a decrease in the relative quantity of organisms present.

One would naturally not expect the increase in the total number of positive specimens to be large, since a considerable number of specimens are undoubtedly submitted on very slight suspicion, thus giving a large percentage of negatives. On the other hand a diagnosis made earlier than was hitherto possible, even if on only a very few samples, is of considerable value to the persons concerned, in the light of our present knowledge.

If a small amount, from 1 to 2 per cent, of a 10 per cent aqueous solution of caustic soda is added previous to the shaking, digestion of the mucous takes place very quickly, rendering the smearing, etc., still easier. Such digestion is necessary if the sputum samples be sedimented.

B. The sedimenting of sputum.—The problem of properly sedimenting the sputum in the original bottles was far more easily solved than that of shaking. Any Babcock machine having a speed of 1,200 revolutions per minute or over does fairly well, provided the sputum bottles used are small enough to fit into the Babcock cups. More uniformly satisfactory results can be obtained with a speed of 2,000 or over. In the machine used by the writer the cups are held in place by trunnions which fit into the slots in a metal head or pronged disc. The regular cups can thus be taken out and special cups of the exact size to receive the sputum bottles inserted.

Lack of time has prevented the presentation at this time of some of the minor details of the method, such as the most advantageous amount of digestive solution to add, the speed, and length of time necessary to obtain the best results. It is the intention of the writer

to determine these points and to secure evidence as to the relative efficiency of this as compared with ordinary methods of examination. Enough has been done to indicate that very satisfactory results may be obtained.

II. A COLLECTION CASE FOR RABIES SPECIMENS.

Rabies first developing in the western part of Massachusetts in the fall of 1905, and becoming epidemic in Boston in the spring of 1906, it became necessary to have some convenient way of severing the head of the supposed infected animal at the spot where it was killed and of conveying the same back to the laboratory. The following answers the purpose well:

A tin-lined copper box 18 inches long by 8 inches wide and 9½ inches deep is divided off at one end into two small compartments. These two compartments are made by running a partition 3½ inches from the end of the box and a second lengthwise partition 3½ inches from the front of the box. The smaller space is designed to carry a one-pint bottle of 5 per cent carbolic acid. The second small compartment is a convenient place for rubber gloves, a bottle of chloroform, etc. The remaining large compartment will easily hold the head of a Newfoundland, St. Bernard, or other large dog. The hinged cover of the box is heavily reinforced and fits down upon a wired edge. On the inner part of the cover are metal clamps, holding a saw and dissecting knife. When closed the cover is held in place by two sliding clasps and a hasp and padlock. A tubular brass handle is firmly fastened to the cover. The complete case is of suitable size and weight for easy transportation. As every part of the box is of metal it can be sterilized by placing on a piece of sheet iron over a two-burner gas stove, filling the box partly full of water and boiling.

When many cases are to be examined, the convenience of such a box as that just described is evident. An untrained laboratory assistant may be sent out with the box, the head of the suspected animal removed, placed in the box, and brought back to the laboratory in public conveyances, if necessary, without the delay of waiting for the express, and without the necessity of disposing at the laboratory of the rest of the carcass.

PLATE 1.

A NEW MOSQUITO CAGE.

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THE mosquito problem is becoming an important one throughout practically all of the western hemisphere, as well as in large areas of Europe, Asia, Africa, and Australia. I do not refer alone to that portion of North America lying between the thirtieth parallels of north and south latitudes. In these regions the normal habitat of the *Stegomyia fasciata*, endemic and epidemic foci of yellow fever make the problem not only important, but urgent; in fact, a large part of the energies of sanitary administrations in Cuba, Mexico, and the Gulf states of the United States, must be directed toward the destruction of mosquitoes and the prevention of their breeding. There are evidences, however, that this problem, now mainly the problem of the hygienist of the Stegomyia area, must be met also by the hygienists of all areas where diptera normally or occasionally occur. Mosquitoes are now entering public hygiene in the United States, not alone in the relatively insignificant "malaria area," in the rôle of "unsanitary nuisances."

Although all varieties of the common Culex and Anopheles have been proven to convey disease, the people of Northern American latitudes are more directly interested in the problem from the standpoint of public comfort. In certain areas, notably on the Atlantic coast of New Jersey and New York, the mosquito is an important economic problem, and its extermination in these states has repaid many fold the expenditure of public money through increased value of the land. The extermination of mosquitoes over any large area, or upon any extensive scale, requires careful study and a definite plan of campaign. An absolutely essential preliminary, is a geographical survey of the area involved, to determine the varieties of mosquitoes to be dealt with, and their distribution. In fact the sanitary laboratory in the near future will have to make studies of variety and distribution of mosquitoes with the same care and attention as is now given to pathogenic organisms. For this purpose it is necessary to collect,

raise, and observe mosquitoes in the laboratory. The study of mosquitoes includes the collection of eggs or larvae, hatching in water, and the preservation and observation of the imagines in suitable cages. The cage here described was devised with the object of facilitating the raising of mosquitoes and their observation during hatching, and in the imago stage. As a preliminary to experiment, observation, and diagnosis of variety, eggs or larvae must be collected, and transferred to hatching-jars. The larvae may be collected by means of a fine net passed through any pond which may breed mosquitoes, well below the surface of the water. Eggs may be easily recognized by a careful observer, and transferred by means of a spoon. The eggs or larvae are transferred to a hatching-jar filled with rainwater until they have fully developed.

The usual method of developing imagines is to hatch the eggs or larvae in jars protected with netting, and to transfer each adult insect separately by means of a short test-tube to cages of wire netting. In order to avoid the laborious handling of each single insect, to avoid loss during transfer, and to facilitate observation and experiment, the larvae jars have been combined with the imago cage in the manner illustrated in the drawing.

Description of the cage.—The cage is of wood, divided into three compartments by partitions. The base is made of a single piece of wood and supports the two vertical wooden partitions and two end pieces. A floor separates the larvae jars from the imago compartments. The top of each imago compartment is closed by a piece of plate glass, held lightly by screws. The sides of the imago portion of the cage are closed by wire and cloth netting (a double layer) held in place by brass upholstery tacks.

The two vertical partitions divide the space between the base and floor into three compartments, each made to fit accurately one of the larvae jars, so that the latter can be slid easily in and out of their compartments without allowing any interval through which the insects can escape when the jar is in place. Each larvae jar communicates with its imago compartment by an opening slightly smaller than the mouth of the jar. Through this opening the imagines pass upward into the netting cage as soon as they undergo their metamorphosis.

The partitions and ends of the cage are supported above by two longitudinal wooden strips each 1½″ wide, mortised into the partitions and ends. All of the wood used in the construction of the cage is ½″ pine.

As it is important for the larvae jars to fit accurately, the wood should be well seasoned and given a thorough rubbing with an oil filler.

The cage, including the netting, should be painted inside and out with a white enamel paint, which will add materially in observing the mosquitoes.

The plates inclosing the tops of each compartment are a good quality of plate glass, ⅛″ thick, and bored for from three to six 0.2″ screws.

The two end plates are $9\frac{11}{16}$" by $8\frac{3}{16}$". The plate closing the central compartment is $9\frac{4}{16}$" by $8\frac{3}{16}$", and the feeding plate of the same dimensions.

The full size of the cage is, height 18 inches, width 8 inches, length 28 inches. The larvae jars are $2\frac{1}{2}$" high, and the diameter of their mouths $4\frac{1}{2}$". Their compartments correspond in height, but are somewhat larger in the other dimensions. A space is thus allowed on the floor of the imago compartment upon which the mosquitoes can rest, and upon which their food (dates, bananas, sugar, and water, etc.) is placed in small butter plates.

Feeding-plate.—If it is desired to propagate mosquitoes through several generations, or to persuade fecund females to lay, or to carry

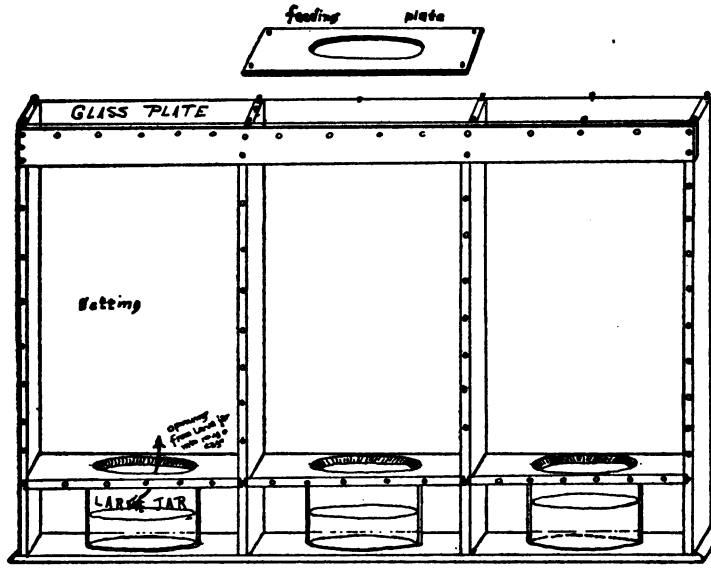

FIG. 1.

out any line of experiments in diseases for which the mosquito is the intermediate host, provision must be made to furnish the impregnated female insects with blood. This is absolutely essential in the case of the Stegomyia, as the female will not lay fertile eggs (if at all) without the previous ingestion of blood, preferably from a human source.

The usual method of furnishing blood to insects in cages, is to provide a special sleeve of netting, protected with a draw-string through which the arm can be introduced into the cage. There are two serious objections to this method. One is that the string produces folds in the netting into which mosquitoes will frequently squeeze themselves

and die, and the other is the impossibility of observing the insects during the act of sucking blood. In the present cage the netting sleeve is replaced by a feeding-plate. This plate is of glass with a central elliptical opening made to fit the forearm of the experimenter at its largest part. A paper or cardboard pattern should be made for the feeding-plate and for the three plates closing the top of the compartments, to show their size, the position of the screw holes, and the position of the central opening in the feeding-plate.

To make the pattern for the feeding-plate, a piece of paper or strathmore board should be cut to the exact size of the plate and the center marked by means of two diagonals. The major and minor axes of the forearm should be determined by means of a pair of compasses or calipers. Draw the major and minor axes intersecting the center at right angles to the sides and with two pins attached to a piece of string, the length of the major axis, draw the ellipse. The pins should be placed equidistant from the center, at such a distance that the curve will just intersect the ends of the axes.

The feeding-plate now in use in the cage was bored and cut to fit the pattern of the forearm by a Baltimore firm dealing in plate glass, but it could probably be made more economically by etching with hydrofluoric acid. In this case it could easily be made an exact fit of the forearm of the experimenter. In this case the ellipse could be described as already stated. After taking the axes of the forearm and describing the ellipse, the forearm may be measured with a piece of lead tape, drawn over the ellipse, and the pattern cut out on a piece of paraffined paper. By pouring hydrofluoric acid upon the opening in the paper and using if necessary several pieces of paper, a hole can be etched which will fit accurately the arm from which the pattern was made.

Method of using the feeding-plate.—The feeding-plate may be used, when it is necessary to supply the impregnated females with blood, in the following manner. This involves the substitution of the feeding-plate for the plain glass plate closing the top of the compartment. To do this the screws are removed from the top plate and the feeding-plate placed against its edge and gradually pressed against the top plate until it has replaced the latter, sliding off at the same time the top plate. The opening in the feeding-plate is closed during this manipulation by one of the covers ground to fit the top of the larvae jars. When the feeding-plate is *in situ* and lightly fastened with screws, the cover is removed, and the forearm inserted. If the opening does not fit accurately a piece of gauze is wrapped around the forearm below the elbow. This glass plate allows an uninterrupted view of the females during blood-sucking.

The cost of the cage with the glass plates is about $15. The largest part of the cost is in the glass plates, but the increased facility of observation more than compen-

sates for their cost. These plates cost in Baltimore $1.50 each for the plain plate-glass top plates, and $5 for the feeding-plate. The cost of the latter is mainly in the feeding-opening, as the boring and cutting of these plates is a difficult and delicate operation.

The larvae jars are the cylindrical jars with ground covers used for instruments and dressings, and cost 50 cents each. Their size is 2½" by 4½". The wooden foundation may be made by any intelligent carpenter. This portion of the cage cost $2.50. The netting can be applied in half an hour by anyone. An inner layer of fine mesh-cloth netting and an outer layer of wire netting were used in this cage.

The glass feeding-plate will be found especially valuable in removing adult insects from the cage, as it permits binoptric vision, which is very difficult if netting or a gauze sleeve is used. There being no netting in the top of the cage, a full and unobstructed view of the imagines is always available. The combination of the larvae jars with the imago cage relieves the experimenter from any close care and observation other than the maintenance of a proper temperature and the supplying of the imagines with food and blood when necessary.

The larvae jars may be removed from the cage at all times, excepting when the pupae are undergoing metamorphosis. By placing the jars upon a white or neutral surface, nearly all the details of structure necessary for diagnosis may be observed without magnification.

The total weight of the cage, including water in the jars, is less than ten pounds, and it can be easily moved about for observation and demonstration.

With the cage made in three compartments, as in the experiments for which it was devised, two separate methods of treatment and one control were provided for, but any number of compartments can be constructed, or a unit plan may be employed.

THE ACTION OF SUNLIGHT UPON BACTERIA WITH SPECIAL REFERENCE TO *B. TUBERCULOSIS.*

JOHN WEINZIRL.

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INTRODUCTION.

THE action of sunlight upon living bodies has always been a problem full of interest to the biologist, and even to the chemist and the physician. The well-known effect upon green plants in which carbon dioxide from the air and water from the soil are gradually changed, through the influence of sunlight, into starch, cellulose, and sugar, naturally led many to believe that sunlight exerted an important influence upon other life as well. With respect to the animal species we know comparatively little, only the lower members, as the protozoa, having been extensively studied; on the other hand, the lower plant forms, especially the colorless plants, early received systematic investigation. The bacteria received the most attention, undoubtedly because of their relation to disease and to national and household economy. The new methods of work which bacteriology originated promoted this sort of investigation; as a result, we possess today an extended literature upon the question of the action of light upon the bacteria. This action is described as bactericidal, the non-spore-

forming species being killed in about one and one-half hours in summer, and two and one-half hours in winter.

One phase of this subject appears, however, to have received little or practically no attention; this is the effect that light exerts upon the bacillus of tuberculosis. When it is recalled that tuberculosis carries off one-seventh of the human race, not to mention the accompanying suffering and financial sacrifices that its long-drawn-out character entails, it appears that this organism should have received early and abundant attention. The cause of this neglect is not difficult to understand. In the first place, the tubercle bacillus is a slow grower when compared with most other bacteria. A day or two will give satisfactory growths with many, while the tubercle bacillus requires two to four weeks. In the second place, tubercle bacilli will not grow on the ordinary media, but require proteids such as coagulated blood serum or egg albumen. The temperature, too, must be held rather closely to 37° C., and the moisture must be carefully conserved. Taken together, these facts have evidently deterred workers in this field from employing the tubercle bacillus.

The question of the action of sunlight upon *B. tuberculosis* was taken up by the writer in the summer of 1905. It was soon found, however, that the conventional methods for testing the action of sunlight upon bacteria were not feasible in working with *B. tuberculosis;* and, indeed, this is another reason why so little work has been done upon this important organism. In attempting to determine a suitable method, it was found that the slow-growing character of the culture made it unsuitable for the rapid testing of new methods; other bacteria were employed for this purpose, *B. coli* answering the purpose admirably because of its vigorous growth, its heavy cloudiness in bouillon, its resistance to desiccation, and, more especially, the readiness with which it could be identified when occasion demanded. Consequently this organism has been used in a large part of the work, and the report made to include these tests. As will be seen presently, this appeared most desirable, for the application of new methods required a comparative study, and this study at once showed certain defects in all the data that had heretofore been gathered. Before proceeding farther, we may with advantage review these data briefly; not that they are few, for they fill many pages, and a simple bibliog-

raphy would include perhaps a hundred titles; nor, indeed, because they are unimportant, for they have served as necessary stepping-stones in our progress.

For bibliographies, the reader is referred to the publications of Marshall Ward, Dieudonné, and Raum. Here only the epoch-making researches, and such as bear directly upon the present line of work, need be considered. These will be presented in some detail in order that direct comparisons may be made with the work presently to be recorded.

HISTORICAL REVIEW.

A. *Action of light on bacteria in general.*—The fact that light exerts a detrimental influence upon the colorless plants must have been recognized at an early date; it was most natural, therefore, when bacteriological methods were sufficiently developed, that considerable attention should have been paid to this group. Within a decade after Koch's discovery of the solid media, many of the important species were tested as to the action of sunlight upon them, and the results recorded. The past decade has added little that is new to this field, save the testing of X- and Radium rays.

The earliest work, preceding even the days of solid media, is that of the now classical investigations of Downes and Blunt.[1] They were, indeed, the first workers in this field. They exposed broth cultures containing a mixture of organisms, and observed a decided inhibitive action. Days or even months were required to sterilize the cultures.

Another early worker in this field was Arloing.[2] He used cultures of anthrax, and came to the conclusion that if spores were present they were not killed out until 25 to 30 hours had elapsed. In an earlier trial, broth tubes containing spores, which had been exposed for two hours in July, remained sterile. If allowed to germinate, 25 to 30 hours were required to kill.

Using one of his Tyrothrix forms which produced spores, Duclaux[3] found that in bouillon tubes:

after	5 days'	exposure	1	out of	3	remained	sterile
"	1 month's	"	2	" "	3	"	"
"	2 months'	"	3	" "	3	"	"

When micrococci were employed[4] the time required to kill them was decidedly less, not more than 40 days in the spring time, or 20 days in summer. If exposed in a dry state, eight days in spring and two to three days in summer sufficed to kill.

Marshall Ward[5] also worked with anthrax, but substituted agar plates for bouillon tubes. Gelatin was tried, but this medium melted in the sun, and so gave uncertain results. His agar plates were covered by a black stencil. The results were not very definite, and varied with the brightness of the days. In March, exposures of one-half

[1] *Proc. Roy. Soc. Lond.*, 1877, 26, p. 488; and 1878, 27, p. 199.
[2] *Comp. rend. de la Acad. Sci. Paris*, 1885, 100, p. 378, and 101, pp. 511, 535.
[3] *Ibid.*, 100, p. 119.
[4] *Ibid.*, 101, p. 395.
[5] *Proc. Roy. Soc. Lond.*, 1890–94.

to one hour inhibited the growth; and one to one and one-half hours killed. He tested the action of the various colored rays, with the general result that the more refrangible (green to violet) rays showed germicidal powers upon both spores and bacilli.

Working about the same time as Ward, we find Buchner,[1] whose classical demonstration of the sun's bactericidal action on a typhoid agar plate is so well known as scarcely to require mention. The plate was covered with black paper through which the letters of the word "typhus" were cut; this plate was exposed to direct sunlight and then developed in the dark. In the letter openings the bacteria were killed, and when the paper was removed, the letters stood out boldly.

In another experiment he made an aqueous suspension of *B. coli* containing 100,000 germs per c.c. After exposing to direct sunlight for one hour, the suspension was found to be sterile.

Dieudonné[2] experimented with *B. prodigiosus*, and *B. fluorescens putidus*, especially because they were supposed to be highly sensitive to light-action. He found that in agar and gelatin plates, one and one-half hours of exposure kill the bacteria in March, July, and August; while two and one-half hours were required in November. Repeating the work with *B. coli*, *B. typhosus*, and *B. anthracis*, substantially the same results were obtained. He also investigated the effect of the different rays by the use of colored solution screens, and by the prism with an arc light. Both confirmed Ward's work. As to the question whether the action was primarily upon the medium and so indirectly upon the bacterium, or upon the organism direct, he believed the latter was mainly the case, although the medium was somewhat changed.

Kruse[3] worked more especially upon secondary questions, such as the effect of the different colored rays, using colored solutions for his screens; also the influence exerted by the presence of gases, such as hydrogen and oxygen. In oxygen the bacteria, when exposed to sunlight, were killed in three hours, while in hydrogen they were still living after six or seven hours. He also tested the action upon the culture medium; while this was affected, the growth being somewhat inhibited, still this was not sufficient to explain the action of sunlight upon bacteria.

Recently some experiments were made at Lawrence, Mass., by Clark and Gage.[4] The typhoid and colon bacilli were employed, watery suspensions of fresh cultures were made, and 1 c.c. of the suspension was placed in a petri dish which was then exposed to direct sunlight. After exposing a definite time, agar was added to the water and the plates incubated. In 10 to 15 minutes' exposure, 95 to 99 per cent of the typhoid germs were killed, and 96 per cent of the colon germs. To destroy completely all life, the time varied from one-half hour up to four hours. These tests were apparently not repeated except in a modified form in one or two instances.

B. *Action of light on B. tuberculosis.*—Coming now directly to the effect upon the tubercle bacillus, we have scanty data to review. The earliest observations were made by Koch whose remarks at the Berlin Tuberculosis Congress, 1890, evidently made from general observations upon this subject, have found their way into so many articles and textbooks as follows:

"The tubercle bacillus is killed quite rapidly by light. A few minutes' to several hours' direct sunlight kills. Diffuse sunlight, though slower, gives the same result. Tubercle cultures set by the window die in five to seven days."

[1] *Centralbl. f. Bakt.*, 1892, 11, p. 781.

[2] *Arb. a. d. kais. Gsndhtsamt.*, 1894, 9, pp. 405, 537.

[3] *Ztschr. f. Hyg.*, 1895, 19, p. 313.

[4] *Rep. Mass. State Board of Health*, 1902, p. 245.

As a result of these remarks, a general impression seems to have gone out that the tubercle is exceedingly sensitive to the influence of light, a point to which reference will be made later.

Frequent reference is also made to the work of I. Strauss,[1] who, after quoting Koch's remarks as given above, says:

"I have been equally able to assure myself that some cultures with abundant human and avian bacilli, developed upon the surface of glycerin bouillon in glass flasks, were killed after having been exposed upon a balcony for two hours to the rays of the summer sun. Besides these flasks I exposed to the sun some cultures previously dried in thin layers on some glass plates; already, at the end of half an hour, they had lost their vegetative function and their virulence."

The work of Migneco[2] also requires notice, although it is of a limited character. He smeared sputum, known to contain numerous tubercle bacilli, upon various kinds of cloth, hung the cloths so as to be exposed to sun and wind, and after various intervals of time, strips (2×5 cm.) were cut off and inoculated subcutaneously or intraperitoneally into guinea-pigs. He concludes that sunlight has a detrimental effect upon tubercle, as well as upon other bacteria, and that upon linen or woolen cloth it can withstand sunlight not more than 24 to 30 hours, provided that the layer of sputum is not too thick; also, the virulence of the bacilli is greatly weakened after 10 to 15 hours.

A number of men have carried out practical tests similar to the above, but the work scarcely requires mention here.

C. *Observations on recorded data.*—Enough has been said to show the strictly limited character of the experiments, and the totally inadequate data for any accurate conclusion to be drawn from them, so far as tubercle is concerned. It will also be observed that in all the experiments (save Migneco's), the various kinds of bacteria were exposed under glass, i. e., in glass vessels. It is a fact well known to physicists that glass reflects and absorbs a large proportion of the sun's rays, depending upon conditions. If the light strikes the surface quite obliquely, then by far the larger portion is reflected; if it strikes vertically, then a considerable amount is absorbed by the glass itself. Again, the bacteria tested have always been planted in or upon some medium (one experiment of Strauss's excepted). These media are always slightly colored or clouded, and consequently absorb even a larger proportion of the light than the glass. In some experiments, the heaping-up or clumping of the organisms, as in naturally grown cultures, or in sputum smears as used by Migneco, the data are totally invalidated save from a most limited point of view. Taken together, these various factors have discounted the effectiveness of sunlight as a germicidal agent, varying from a fourth to perhaps nine-tenths, or even more in some cases. These shortcomings in methods of work the writer has succeeded in completely overcoming, but only after a number of months of laborious work.

Hence the desirability of more adequate and accurate data concerning the action of sunlight upon the bacillus of tuberculosis goes unquestioned. It would seem, too, that a general reopening of the question of the action upon bacteria in general is not only justified, but is most highly desirable, especially in view of the rapid strides that sanitary science is making at the present time.

[1] *La tuberculose et son bacille*, 1895, p. 220.

[2] *Arch. f. Hyg.*, 1895, 25, p. 361.

A STUDY OF METHODS

Even at the beginning of the work, or rather as a result of some work done along this line in New Mexico, it was recognized that new methods were necessary, if the data are to have the highest value. This study of methods took two directions: first, an attempt to find a method that could be used with *B. tuberculosis*, since the methods usually employed fail utterly when applied to this organism; secondly, an attempt to minimize or eliminate completely the absorption factor of the interposed glass and media. These two lines of work were developed simultaneously, and it will not be convenient to separate them in the discussion, for, as will be seen, each helped to solve the other.

A. *Exposure of organism on media.*—As frequently happens, this work was begun at the most difficult point, viz., the methods for tubercle. Dorset's egg medium[1] furnished the most ready and satisfactory method of growing pure cultures of tubercle, and consequently this was selected for the work at hand. The plate method of exposure as commonly practiced was not suitable, because of the rapid desiccation incident to incubation. The most natural suggestion was to employ sloped test-tube cultures, similar to agar slopes, and expose these. Dorset's glycerine-phosphate agar was tried in the same way.

On August 19, 1905, a large number of cultures, made as suggested above, were exposed to direct sunlight for one-half, one, two, three, and five hours respectively. Two strains of tubercle bacilli were used:

Culture No. 101,[2] human type, was isolated at the local laboratory from human sputum, and was of fully average virulence.

Culture No. 110, from bovine source, was isolated by Dr. E. L. Baldwin of Saranac Lake. The virulence of this bacillus, when tested by the writer, was certainly not greater than that of No. 101, and was considerably below what is usually considered as characteristic for the bovine variety.

In this connection the descriptions of the additional varieties subsequently used may also be given.

Culture No. 102, was obtained from the Bureau of Animal Industry, U. S. Department of Agriculture, where it was used in making tuberculin. It possesses very low pathogenic powers, and grows rapidly and abundantly on egg medium.

Culture No. 113, avian, was isolated by the writer from a tuberculous hen in July, 1905. It grows more sparingly than the two human cultures, the colonies being more restricted and elevated.

[1] *Bull. Bur. Anim. Indus.*, 1904, 52, Pt. I.

[2] The numbers are those in use at the local laboratory, and have no special significance.

These bacilli, taken from young cultures, were inoculated upon the sloped media in test-tubes, and exposed to the direct rays of the sun on a wire support raised about eighteen inches from the floor. In this and all subsequent experiments the attempt was made to keep the sun's rays vertical to the surface of the medium; this was impracticable except when employed as an approximation toward the ideal. In addition to the egg and agar cultures, a watery suspension was exposed in a test-tube similarly to the others, and cultures made at intervals. Another variation consisted in rubbing the bacilli on small strips of sterile cloth, exposing these in a petri dish, and then inoculating the cloth and bacilli into an egg tube.

Substantially the same series of experiments was repeated on September 8, substituting paper for the cloth, however, and inoculating this upon egg. The results of these experiments are embodied in Table 1 below:

TABLE 1.

EFFECT OF SUNLIGHT UPON CULTURES OF *B. tuberculosis*.

EXP. NO.	DATE	NO. OF CULTURE	MEDIUM USED	RESULT OF EXPOSURE (HOURS)	
				Growth	No Growth
1	Aug. 19, '06	101	Egg	½, 1, 2, 3	5
2	" 19, '06	101	Agar	½, 1, 2,	3, 5
3	" 19, '06	101	Aqueous suspension	½	1, 2, 3, 5
4	" 19, '06	101	Cloth	½, 1, 2, 3	5
5	Sept. 8, '06	101	Egg	½, 1, 1½, 2, 3	.....
6	" 8, '06	101	Aqueous suspension	½	1, 1½, 2, 2½, 3
7	" 18, '06	101	Paper	½	1, 1½, 2, 2½, 3
8	Aug. 19, '06	110	Egg	½, 1, 2, 3, 5	.....
9	Sept. 8, '06	110	"	½, 1, 1½, 2, 3	.....
10	" 8, '06	110	Paper + Moisture	½	1, 1½, 2, 3

The data in this table showed a number of interesting points:

a) The method of exposing test-tube cultures is plainly unsuited to this purpose. This is readily understood when it is recalled that many of the bacilli are worked into the egg during the process of inoculation, and are consequently materially protected. The cylindrical surface of the tube tends to concentrate the light in a line and thus produce unequal intensities upon different portions of the surface of the medium. Consequently, this method was immediately abandoned.

b) The method of exposing watery suspensions appeared quite promising, but it was open to the objection of unequal distribution

of light, which is not wholly remedied by placing the suspensions in potato or petri dishes. In addition, there was a marked tendency for the culture to sediment.

c) The method of spreading the culture on cloth and subsequently inoculating upon egg was also unsuitable, for the reason that the culture worked into the interstices of the cloth and so received protection. Data thus obtained are quite worthless, since we have no means of knowing how much of the effect to ascribe to sunlight and how much to the factor of protection.

d) The method of spreading the pure culture upon sterile paper slips and then exposing them in petri dishes gave the same results as the watery suspensions, and at the same time eliminated the objectionable features of that method, i. e., the unequal distribution of light. Accordingly, this method was temporarily adopted in the work, and considerable data collected, before any advance was made.

B. *Exposure of organism on strips of paper under glass.*—The strips of paper used were about 1 × 5 cm. area; they consisted of ordinary, sized paper and were sterilized by heat before using. Such strips of paper are difficult to inoculate when placed in a glass vessel; but this difficulty can be overcome by placing a piece of cloth in the bottom of the dish and laying the paper upon it; the cloth holds the paper in place both during the inoculation and the exposure. Watery suspensions spread very readily upon such sized paper, serving better than many materials subsequently tried.

In applying this method, several important questions arose, which require discussion:

a) *How does the quantity of culture inoculated upon the paper influence the result?* An early instance of the possible variation in result due to this factor was observed. Among a series of cultures, exposed September 8, were two sets on paper slips: one set showed that tubercle was killed between one and one-half and two hours; the other set between one-half and one hour. At that time the method of inoculating the paper slips was to rub upon them some of the culture by means of a stiff platinum wire. Plainly, the amount of culture transferred to the paper varied within wide limits, even when special care was exercised. Portions from different parts of the culture tube would spread very differently, and necessarily required

different periods of exposure to kill, as shown by the instance cited above. Other instances of such variation were observed, but the details need not be given, for the point is perfectly plain. Suffice it to say that steps were immediately taken to remedy this defect. These steps were as follows:

(1) Instead of taking the culture direct, a suspension in plain water was first made, then a small loopful of this suspension was spread upon the sterile paper slips. With most species of bacteria, this method works quite satisfactorily, but this is not always the case with tubercle. Cultures of tubercle vary so widely in their physical condition, depending upon their age and the medium employed, that all grades ranging from a soft, friable to a hard, resistant mass are obtained. These do not work up equally well into a homogeneous suspension, but are apt to produce flocculi which may readily be carried over to the paper. It is obvious that the germs within or under such a lump are protected to a considerable degree from the influence of sunlight. The effort to overcome this difficulty led to the second step:

(2) The filtration of the suspension through glass wool. Necessarily this must be done under aseptic conditions to prevent contamination. The suspension so obtained is much more uniform in character, all the gross lumps and fatty flocculi being removed. It does not completely remove the microscopic lumps consisting of 10 to 50 bacilli, and to break these up still further it is advisable to employ a shaking machine for some time. However, quite uniform results can be obtained without the additional shaking.

b) How is the inoculation of the media with the cultures on paper to be accomplished? This was done in a simple and surprisingly satisfactory manner by placing the paper by means of a sterile forceps on the egg slope. At first an attempt was made to dislodge the bacilli from the paper, but it was soon found that the culture grew admirably upon the paper if it was carefully pressed upon the medium and sufficient fluid was present to moisten the paper. The nutrient medium filters through the paper and nourishes the bacteria, which grow in a wholly satisfactory manner, as shown in Pl. 2. The method is applicable to agar slopes also, if care is taken to have some expressed water present; but obviously, old culture media work unsatisfac-

torily. When experimenting with bacteria which grow well in broth or other liquid media, the method becomes the acme of simplicity; the slip of paper is dropped into the tube of bouillon or milk, placed in the incubator, and the characteristic growth phenomena noted.

c) Is it possible to diminish the absorption of light by substituting a more suitable medium for the glass? Pure quartz plates would absorb less light than common glass, but such plates are expensive and practically out of the question. Gelatin films and mica plates next suggested themselves, and these were accordingly tried. On October 28, cultures of Tubercle No. 101 were exposed on paper under these covers, and also under glass. The results showed that:

1. Under glass, tubercle was killed between 0 and ½ hrs.
2. " mica, " " " " 1½ " 2 "
3. " celluloid, " " " " ½ " 1 "

Apparently there was no advantage to be gained by the use of either mica or celluloid. Before the test was repeated, the problem was solved by rendering it non-existent as will be shown later. The whole question might well have been ignored, but for the fact that it illustrates nicely one of the steps in the progress of the work.

d) What part of the bactericidal effect is due to desiccation? Considerable indirect evidence, gained from the earlier experiments, indicated that it was a minor, if not a negligible, factor.

A direct experiment, made December 30, gave the following results:

TABLE 2.

	Organism	Moisture Added	Result of Exposure (Min.)	
			Growth	No Growth
1.....	*B. coli*	−	..	3, 6, 10, 15, 20, 30
	"	+	3	6, 10, 15, 20, 30
2.....	*B. typhosus*	−	..	3, 6, 10, 15, 20, 30
	"	+	..	3, 6, 10, 15, 20, 30
3.....	*M. tetragenus*	−	..	3, 6, 10, 15, 20, 30
	"	+	..	3, 6, 10, 15, 20, 30
4.....	*B. diphtheriae*	−	..	3, 6, 10, 15, 20, 30
	"	+	..	3, 6, 10, 15, 20, 30

It should be noted that the above exposures were made on an unusually clear day, which accounts partially for the short time required to kill the cultures. While, in one instance, the moisture seems to have prolonged the life of the organism, yet, taken as a whole,

the total time involved was so short as to make it quite doubtful whether this was a determining factor.

In another experiment, April 7, an attempt was made to exaggerate conditions by surrounding one set with conc. sulphuric acid, thus rendering the air quite dry, while the other set had sufficient water added to cause a film of moisture to gather under the cover. Under these conditions, both sets were killed within five minutes, the shortest time of exposure.

It appears highly probable, therefore, that the presence of moisture is neither instrumental in killing, nor in greatly prolonging the life of the cultures. Indeed, as we shall see, the method finally adopted totally removes this question from the main problem.

e) *Does the character of the paper used influence the effect of sunlight in killing the culture?* It was stated at the beginning that the paper used was a common, sized writing-paper. It is conceivable that in the process of manufacturing, especially in bleaching, some antiseptic properties were communicated to the paper. To test this possibility, *B. coli* was exposed to sunlight on eight different substances (March 15) with the following results:

TABLE 3.

Trial	Material Employed	Result of Exposure (Min.)	
		Growth	No Growth
1	Ordinary, sized paper	2, 7	12, 20
2	Filter paper	2, 7, 12, 20	.....
3	Black porous paper	2, 7, 12, 20	.....
4	Parchment paper	2, 7	12, 20
5	Aluminum foil	2, 7, 12, 20	.....
6	Mica	2, 7, 12	20
7	Glass	2, 7, 12	20
8	Wood	2, 7, 12, 20	.....

These results appear somewhat surprising at first; but, upon analysis, the reasons for the differences observed are quite plain. The filter paper, the porous paper, and the wood (a soft pine) are sufficiently porous to permit the individual bacilli to enter with the water and thus become sheltered; the aluminum foil, on the other hand, did not permit the making of a uniform spread, the culture drying in patches, which caused a heaping-up effect with consequent protection.

Similar experiments with the same organism gave the following:

TABLE 4.

TRIAL	DATE	MATERIAL EMPLOYED	RESULTS OF EXPOSURE (MIN.)	
			Growth	No Growth
1.....	March 16	Paper previously used	2	5, 10, 15, 20
2.....	" 16	Parchment	2, 5, 10, 15, 20	.....
3.....	" 16	Mica	2, 5, 10	15, 20
4.....	" 16	Glass	2, 5	10, 15, 20
5.....	" 17	Paper previously used	3, 6	10, 15, 20
6.....	" 17	Parchment	3, 6, 10, 15	20
7.....	" 17	Mica	3, 6, 20	10, 15
8.....	" 17	Glass	3, 6, 20	10, 15
9.....	" 17	Filter paper	3, 6, 10, 15, 20, 30	.....

The filter-paper test requires no further comment. Glass shows the same phenomenon as aluminum when not absolutely free from grease; this tendency is shown by the erratic results that it gives. The parchment invariably became roughened and warped in the sterilizing process, which renders it unsuitable for the purpose in hand. There is left to consider only the mica. This was a genuine disappointment to the writer, for the culture spread very evenly upon it. Apparently this should be an ideal substance to use and would serve as a fair check upon the paper method. Upon still other repetitions this continued to give erratic results, and the explanation undoubtedly is to be found in the fact that the surface of a small piece of mica is very imperfect, many cracks extending inward from the cut edges; in these cracks the bacteria evidently found shelter.

Thus the tests fell short of their object, and merely proved the inferiority of these substances for this purpose. Sized paper gives a beautiful spread, as compared with these; there is little tendency to creep into pores, for these are filled by the sizing, and the results are as uniform as could well be expected.

The question with which we started is answered in a measure, at least, by the fact that innumerable control experiments always gave growth, even when the culture remained exposed for several days. Again, *B. tuberculosis* grew admirably upon these papers (see Pl. 2), which certainly contra-indicates the presence of antiseptic principles in material quantities. Finally, as will be seen, cultures exposed without the presence of paper are killed in even shorter periods of time.

C. Direct exposure of organisms.—Thus far, the object has been to minimize the absorption factor, and in this way approach more

closely to the true disinfecting action of sunlight. A very material advantage has been gained, *the exclusion of the nutrient medium and the accompanying absorption.* Incidentally this affords another advantage also, viz., the elimination of possible by-products which may be formed in the medium, such as hydrogen peroxide, ozone, organic peroxides, etc., which might aid in killing the cultures and thus complicating conditions, and rendering the action of the sunlight still more problematical. Indeed, it was not certain that any of the disinfecting action could positively be ascribed to the direct action of the sun's rays. To have eliminated these side reactions and at the same time have a method that is applicable in general, for *B. tuberculosis* included, seemed for a time to present the limit of perfection attainable. Nevertheless, the problem of the elimination of all absorption to obtain the true effect of sunlight was not forgotten.

This problem was attacked at the most difficult point. It was known that the tubercle bacillus could be exposed directly, and by use of a suspension which could be inoculated into animals the contaminating factor would be practically negligible. Accordingly, this experiment was tried. A suspension (heavier than usual), of tubercle No. 101, was allowed to evaporate in the bottom of a petri dish, and exposed, uncovered, to the direct rays of the sun. Four or five drops of the suspension were used to insure sufficient material for the inoculation. At the same time, two of the watery suspensions were also exposed in petri dishes, one being covered and the other not, the latter to serve for comparison. These cultures were exposed for one-half, one, one and one-half, and two hours, when they, together with controls kept in the dark, were injected respectively into guinea-pigs, the inoculations being intraperitoneally in all cases.

This experiment was unfortunate in two respects; firstly, the day chosen gradually developed into a hazy one, so that the sunlight was much diminished in power; secondly, a number of the test animals died from an epidemic of disease that affected the stock of guinea-pigs on hand. The guinea-pigs inoculated with the cultures exposed for the longest time, i. e., two hours, all developed tuberculosis and died. The living tubercle bacillus was recovered in pure culture from several animals of this series. The fact that the cultures were alive after two hours' exposure is explained by the diminished sunlight, on

one hand, and by the thickness of the film and the tendency of the suspensions to sedimentate, on the other.

Several months later it occurred to the writer that organisms could be exposed without cover, and all absorption of light be entirely avoided. This method gave most gratifying results as follows:

A suspension of *B. coli* was made, and a loopful spread in a circle in the bottom of sterile petri dishes. (See Pl. 3.) While exposed to the sun, the dishes were uncovered; when taken in, they were again covered, and a layer of lactose litmus agar, which was melted and cooled to 45° C., was spread over the exposed film. The cultures were placed in the incubator for 24 and 48 hours. The first two sets of plates showed that *B. coli* was killed within two minutes. On March 23, the same method showed *B. coli* to be killed in between two and four minutes. March 31, *B. diphtheriae* was killed in between two and five minutes.

The advantages in selecting *B. coli* in the above and subsequent experiments are these: (*a*) It is not apt to be contaminated with fresh colon bacilli from the air; (*b*) it grows well as 37°–39° C., while the common air forms that might contaminate the plate do not, as a rule, develop at this temperature; (*c*) it grows well on lactose litmus agar, while most bacteria do not; (*d*) it gives red or acid colonies, on this medium, while this would rarely be the case with air germs; (*e*) if any question arose as to whether a given colony was *B. coli* or not, cultures in glucose bouillon fermentation tubes and in milk would decide the matter, in nearly all cases, the proper gas formula being especially helpful in reaching a decision.

That this method, with suitable variations in media, etc., can be applied to all bacteria, goes almost without saying. Glucose agar will be found best adapted to all acid-producing species, in which case the circle or other character used for the film will be most helpful (see Pl. 3); if only occasional colonies appear they should be isolated and subjected to confirmatory tests. Species that require a highly proteid medium, like *B. tuberculosis*, may be planted upon strips of sterile paper, and after exposure inoculated upon egg or blood serum. Necessarily, a sufficient number of cultures must be made so that one or more of each lot for a given period will come through uncontaminated; on a quiet day this is not at all difficult to accomplish.

D. *Comparison of methods.*—In a certain way, the methods employed have been compared with each other; but it will now be advantageous to make a more direct comparison, especially of data secured with this object in mind. For this purpose only three methods have been considered available; viz.: (*a*) the agar-plate method; (*b*) the method of paper cultures under glass; and (*c*) the method of direct exposure of the bacterial films, no glass or other

medium being interposed. The method of exposing suspensions has not been considered because of its numerous disadvantages.

On March 23, an experiment with *B. coli* partly covering these points gave the following:

1. On paper, under glass, *B. coli* was killed in between 11 and 15 min.
2. " glass, " " " " " " " 10 " " — "
3. " paper, no cover, " " " " " 1 " 2 "
4. " glass, " " " " " " " 2 " 4 "

The absorption of light by the glass is considerable, and in this instance prolonged the life of the bacterium considerably beyond that required to kill without any interfering medium.

April 11, another series with *B. coli* gave the following:

1. On glass, no cover, killed within 2 min.
2. In agar plate, glass cover, 25 per cent were killed in 10 min.
   85 " " " " " 20 "
   95 " " " " " 30 "
   100 " " " " " 45 "

These plates are shown in composite in Pl. 3, Fig 2.

A repetition of the above experiment with other bacteria gave the following results:

TABLE 5.

Organism	Date	Time Required to Kill	
		By Direct Exposure	In Agar Plates
B. cholerae suis	Oct. 12, 1906	Between 5 and 10 min.	In 40 min. 10 per cent killed
B. phosphorescens	" 25, 1906	" 1 and 2 "	" " " 95 " "
B. prodigiosus	" 30, 1906	" 5 and 10 "	" " " 70 " "
B. Friedländer	Nov. 7, 1906	" 0 and 1 "	" 20 " 75 " "
B. typhosus	" 9, 1906	" 5 and 10 "	" 40 " 100 " "
			" 45 " 90 " "

The time required to kill bacteria is reduced to at least $\frac{1}{15}$th, and possibly to $\frac{1}{20}$th, of the time required to kill by the old or plate-culture method. These data could be largely extended, but they are sufficient for the present purpose, viz., to show the great advantage possessed by the method of direct exposure. They indicate also the unreliable character of the data thus gathered by the earlier observers, and which have, at most, only a relative value, for only a $\frac{1}{10}$th or $\frac{1}{20}$th of the sun's disinfecting action has been employed in securing them.

E. *Observations on methods.*—Before leaving the matter of methods a number of minor points may appropriately be mentioned here.

a) Apparently most investigators have paid little heed to the meteorological conditions, or have failed to mention the details. In the present investigation, only cloudless sunlight has been employed, unless otherwise stated. The time has been as near midday as practicable, most of the determinations having been made between 11 and 1 o'clock. In this work the accompanying table has been of material service.

TABLE 6.

COMPARATIVE LIGHT VALUES* (EXPRESSED IN SECONDS).

Time of Day	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
8:00.....	10.0	6.0	3.0	2.0	1.7	1.7	1.5	1.7	2.0	3.0	4.0	9.0
9:00.....	4.0	4.0	2.0	1.5	1.2	1.2	1.2	1.5	1.5	1.7	3.0	3.0
10:00.....	2.5	2.0	1.5	1.7	1.0	1.0	1.0	1.0	1.0	1.5	2.0	2.0
11:00.....	2.0	1.7	1.2	1.0	0.7	0.6	0.5	0.7	1.0	1.2	1.7	1.5
12:00.....	1.7	1.5	1.0	1.0	0.5	0.3	0.2	0.5	0.5	1.0	1.5	1.7
1:00.....	1.7	2.0	1.0	1.0	0.7	0.5	0.5	0.5	1.0	1.2	1.5	2.0
2:00.....	2.5	2.7	1.7	1.2	1.0	0.7	1.0	1.0	1.2	1.5	2.0	2.5
3:00.....	4.5	3.5	2.0	1.5	1.2	1.2	1.2	1.2	1.5	2.0	2.5	3.5
4:00.....	9.0	5.0	4.0	2.0	1.5	1.5	1.5	1.7	2.0	2.7	5.0	8.0
5:00.....		60.0	20.0	4.0	2.7	2.2	2.2	2.5	3.0	6.0	20.0	80.0
6:00.....				20.0	15.0	5.0	4.0	5.0	10.0	40.0	70.0	
7:00.....					80.0	20.0	15.0	60.0				

* By Lieutenant S. W. Very, U. S. N. Printed in *Am. Annual of Photography and Photographic Times Almanac* for 1899.

Table 6 gives the relative light values for photographic purposes expressed as time necessary to expose the plate. All the work has been done at times falling between 0.2 and 1 in this table, thus giving the most intense light obtainable. This affords a certain standard for the work; absolute values, if attainable, would scarcely serve a better purpose.

b) During the warmer days the temperature was taken to guard against the possibility of killing the cultures by heat. It is evident that, in the short exposures as finally practiced, temperature is not a determining factor in any case.

c) Young cultures were used for the exposures; these were usually one or two days old, and rarely more than a week old. In the case of tubercle, the cultures varied from one to two months in age. Controls always served as an additional check upon the vitality of the culture.

EXPERIMENTAL RESULTS.

The striking difference in the results obtained by the old methods as compared with those here suggested is best illustrated by a direct comparison of results for specific bacteria.

B. coli.

Dieudonné[1] found that agar plate cultures of *B. coli* when exposed to midday sun were killed in 1 to 1½ hours. Buchner[2] found that aqueous suspensions were sterile after 1 to 1½ hours' exposure.

In comparison with these, the writer found the following:

TABLE 7.

Date	Method of Exposure	Result of Exposure (Min.)	
		Growth	No Growth
March 20, 1906..	On glass, uncovered	.....	2, 4, 6, 8, 10, 15
" 23, 1906..	" " "	½, 1, 2	4, 7, 10
" 23, 1906..	" paper, "	½, 1	2, 4, 7, 10
April 7, 1906....	" glass, "	2	5, 10, 20, 40
" 11, 1906....	" " "	.....	2, 5, 10, 20, 30

The difference between the above results and those obtained by former investigators is most remarkable.

B. typhosus.

Kedzior[3] found that if a 5 c.c. bouillon culture of typhoid was exposed to sunlight, it was not killed in 4½ hours; even a 1 c.c. culture was not killed in 5½ hours.

By direct exposure this organism was killed as follows:

TABLE 8.

Date	Method of Exposure	Results of Exposure (Min.)	
		Growth	No Growth
April 27.....	On glass, uncovered	2, 5	10, 15, 20
May 10.....	" " "	.....	2, 5, 10, 20, 30
" 15.....	" " "	2	5, 10, 20

The results of April 27, are nearer the others than the figures would seem to indicate, for only a couple of colonies remained on the plate exposed for five minutes. When compared with Kedzior's results, it is seen that the time necessary to kill typhoid by direct exposure is reduced to $\frac{1}{18}$ or less. The advantages of the method of direct exposure are evident.

B. dysenteriae (Shiga).

Quite similar to *B. coli* and *B. typhosus* are the results for *B. dysenteriae:*

TABLE 9.

Date	Method of Exposure	Results of Exposure (Min.)	
		Growth	No Growth
April 22.....	On glass, uncovered	.....	2, 5, 10, 20, 30
May 9.....	" " "	.....	2, 5, 10, 15, 20
" 10.....	" " "	2	5, 10, 20, 30

[1] *Arb. a. d. kais. Gsndhtsamt.*, 1894, 9, p. 405.

[2] *Centralbl. f. Bakt.*, 1892, 11, p. 781.

[3] *Arch. f. Hyg.*, 1899, 36, p. 323.

S. cholerae-asiaticae.

In an experiment similar to that with typhoid bacilli, Kedzior found that a 5 c.c. bouillon culture gave a good growth after 4 hours, and a 1 c.c. culture was killed in 5¾ hours.

Direct exposure gives the following:

TABLE 10.

DATE	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
		Growth	No Growth
April 11.....	On glass, uncovered	.....	2, 5, 10, 20, 30
" 27.....	" " "	.....	2, 5, 10, 15, 20
May 19.....	" " glass cover	1, 2	6, 10, 15

B. diphtheriae.

Kedzior also exposed cultures of diphtheria and found them to be killed in 1½ to 2½ hours.

By direct exposure, this organism was killed as follows:

TABLE 11.

DATE	RESULTS OF EXPOSURE (MIN.)	
	Growth	No Growth
March 31.....	½, 1, 1½, 2	5
April 27	2	5, 15, 20
May 10.....	2	5, 10, 20, 30

The Pus Cocci.

No one seems to have experimented with these forms, hence, no comparison is possible. The results obtained by direct exposure are as follows:

TABLE 12.

DATE	ORGANISM	RESULTS OF EXPOSURE (MIN.)	
		Growth	No Growth
April 2.....	*M. pyog. aureus*	3, 6, 10	15, 25
" 22.....	" "	2, 5	10, 20, 30
May 15.....	" "	2, 5	10, 20
April 7.....	*M. tetragenus*	2	5, 10, 20, 40
" 11.....	"	2	5, 10, 20, 30
" 22.....	"	2	5, 10, 20, 30
May 10.....	"	.....	2, 5, 10, 20, 30

In the earlier work two tests were also made with *M. pyog. albus*, but at that time the short period of exposure necessary to kill was not appreciated, and so the results are not very helpful. In the first trial, it was killed within 30 minutes, and in the second within 10 minutes.

B. prodigiosus.

Dieudonné[1] studied this organism because he thought it would be especially sensitive to light. He found it to be killed in between 1½ and 2½ hours in gelatin and agar-plate cultures.

Direct exposure gave the following:

TABLE 13.

DATE	RESULTS OF EXPOSURE (MIN.)	
	Growth	No Growth
April 27.....	.....	2, 5, 10, 15, 20
May 11.....	1, 2	5, 10, 15

It may be worth noting that, on the two-minute plate of May 11, the color production was not diminished, although the number of colonies was greatly reduced.

B. pyocyaneus.

Kedzior worked with this organism and found it was killed in agar plates in between 2½ and 3½ hours. By the method of direct exposure, the writer found it was destroyed as follows:

TABLE 14.

DATE	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
		Growth	No Growth
April 7.....	On glass, uncovered	.....	2, 5, 10, 20
" 11.....	" " "	2	5, 10, 20, 30
May 9.....	" paper, "	.....	2, 5, 10, 15, 20
June 30.....	" glass, "	½, ½, 1, 2	4, 10

Two other chromogens were tried, with the following results:

TABLE 15.

DATE	ORGANISM	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
			Growth	No Growth
April 2.....	*Sar. aurantica*	On glass, direct	3, 6, 10, 15, 25	.....
May 15.....	" "	" " "	2, 5, 10, 20	.....
" 19.....	" "	" " under glass	10, 20, 30, 40, 60	.....
" 28.....	" "	" " direct	60	90, 120
" 15.....	A pink air-micrococcus	" "	2, 5, 10, 20	.....
" 19.....	" "	" " under glass	10, 20, 30, 40, 60	.....
" 28.....	" "	" " direct	60, 90	120

These two organisms show a higher order of resistance to the influence of sunlight than those heretofore considered. (See Pl. 3. B.) Indeed, they constitute a group by themselves, to which, presumably, many of the bacteria found in the air belong. Whether the resistance they exhibit is due to a failure to break up the groups, or to a sporelike condition (so-called arthrospore) of the bacterium, remains uncertain. But, from the nature of the suspensions used, and from the character of the growth in the plates, the writer is inclined to hold the latter view.

[1] *Arb. a. d. kais. Gsndhtsamt.*, 1894, 9, p. 405.

It is fortunate that the pathogenic organisms show no such powers of resistance, or the problem of coping with them would be materially more difficult.

B. tuberculosis.

Coming now to the bacillus of tuberculosis, which is the chief factor in the present investigation, one finds practically no previous tests that are satisfactory, with which to compare the results. Koch's remarks are seemingly based upon casual observation, which, however, indicated that the organism was highly sensitive to light, presumably more so than other bacteria. Strauss came close to the truth in his trial by exposing a film on glass plates, which he found was killed in 30 minutes. Other workers have used sputum with variable results, ranging from 24 to 30 hours, up to days or weeks.

The earlier results when the bacillus was exposed on egg medium have been given; they are relatively high, varying between two and five hours in time necessary to kill; in aqueous suspensions it was killed in ½ to 1 hour. The later results, when no culture medium was used in the exposure, are as follows:

TABLE 16.

DATE	NO. OF ORGANISM	METHOD OF EXPOSURE	RESULTS OF EXPOSURE	
			Growth	No Growth
September 8, 1905....	101	On paper, under glass	½, 1, 1½	2, 3, 4, 5, 6 (hrs.)
" 8, 1905....	110	+moisture, on paper, under glass	½	1, 1½, 2, 2½, 3 (hrs.)
" 8, 1905....	101	" " " " "	½	1, 1½, 2, 2½, 3 (hrs.)
October 28, 1905....	101	On paper, under glass	.....	½, 1, 1½, 2, 2½, 3 (hrs.)
November 11, 1905....	101	" " " "	½, 1, 1½	2, 2½, 3, 4 (hrs.)
" 30, 1905....	101	+moisture, on paper, under glass	10, 20	30, 45, 60 (min.)
December 24, 1905....	101	On paper, under glass	.....	10, 20, 30, 45, 60 (min.)
" 26, 1905....	101	" " " "	½	1, 2, 3 (hrs.)
" 26, 1905....	101	" " " "	.....	10, 20, 30, 45, 60 (min.)
" 26, 1905....	102	" " " "	10	20, 30, 45, 60 (min.)
" 26, 1905....	110	" " " "	.....	10, 20, 30, 45, 60 (min.)
" 26, 1905....	113	" " " "	10	20, 30, 45, 60 (min.)
April 4, 1906....	101	" " direct	5, 10, 15	20 (min.)
" 11, 1906....	102	" " "	.....	5, 10, 15, 20 (min.)

In the work prior to December, the data showing greatest longevity were obtained, as previously noted, by rubbing the pure culture upon the paper and then making the exposure; the December data were secured from aqueous suspensions inoculated upon paper, while those in April and later were films from suspensions, dried on paper and exposed to the sun directly, i. e., without glass or other intervening medium.

Leaving out of account the earlier results, as not being satisfactory, it appears that the method of direct exposure gives as consistent and similar results with tubercle as with other non-spore-bearing bacteria; it is more sensitive than some, but less so than *B. coli*, for example. Too close comparisons are not admissible here, for the time is so short that slight but unavoidable variations may place the result on one side or the other. Apparently tubercle is not especially sensitive to light. This may be only apparently so, for, as was mentioned earlier, homogeneous suspensions are not so readily obtained with tubercle as with other bacteria, and, as a result, clumping, with consequent protection, takes place.

In this connection, another experiment will be of interest. A sample of sputum, containing numerous tubercle bacilli and also cocci, etc., was spread in thin films on papers and exposed under glass in the usual way. In the cultures where development took place, *M. pyogenes aureus* and *albus* were present; but in those that remained free from contamination, tubercle failed to develop. The periods of exposure were 10, 20, 30, 45, and 90 minutes. A repetition of this experiment gave the same results. This would seem to indicate that the tubercle bacillus is not more resistant to the action of light than were the other bacteria that chanced to be present in the sputa.

The details of an experiment made on April 4 will be interesting and instructive, especially in helping to explain the variable results obtained with tubercle, and also its apparent endurance. A watery suspension of a culture of tubercle No. 101 was employed. This suspension was filtered through a layer of glass wool, and subsequently inoculated upon paper strips. These strips were sealed at one end to the bottom of a petri dish by means of hot paraffin. After inoculation, they were exposed, uncovered, to direct bright sunlight between 12:28 and 12:54 P. M. The results are given in Table 17.

It is seen that none of the five-minute exposures was killed; that two out of four of the 10-minute exposures grew, one showing only a single colony; also two from the 15-minute lot showed each a single colony; and that none of the 20-minute cultures grew. Two controls gave excellent growth. Although the suspension from which these inoculations were made was filtered, and a macroscopic exami-

TABLE 17.

RESULTS WITH TUBERCLE, BY DIRECT EXPOSURE

Trail No.	Time of Exposure	Result
1	5 min.	Good growth
2	5 "	Four colonies
3	5 "	Fair growth
4	5 "	Slight growth
5	10 "	No growth
6	10 "	One small colony
7	10 "	No growth
8	10 "	Good growth
9	15 "	Contaminated
10	15 "	One colony
11	15 "	" "
12	15 "	No growth
13	20 "	" "
14	20 "	" "
15	20 "	" "
16	20 "	" "
17	Control	Good growth
18	"	" "

nation showed decided improvement in homogeneity as compared with the original, yet a microscopic examination still revealed occasional clumps of bacilli aggregating 10–50 in number. Undoubtedly it is due to this tendency to clump that tubercle gives such varying results, and a false impression is obtained in regard to its true resistance to sunlight, which is probably not greater than that of the common pus cocci.

An experiment was made with Möller's grass bacillus, which is acid-fast and simulates tubercle very closely, except that it grows at room temperature on common media. In this case it was not killed in 15 minutes, on May 11, at midday. Here clumping was marked, and was accompanied by a corresponding longer period of resistance to sunlight.

It was stated by Koch that cultures of tubercle are killed in five to seven days in diffuse light. This point was also tested, cultures on paper slips being exposed for comparison. The exposures were made directly in front of a north window in the laboratory next to the window-sill, all the cultures being under glass.

In this series the pure culture was rubbed onto the paper slips. The sunlight afforded by a number of days was necessary for these long exposures. While the results are too few for drawing any positive conclusions, they do not indicate any extraordinary sensitiveness to diffuse light on the part of tubercle, for the culture of October 21 required six days of exposure and then was not killed. The cultures

TABLE 18.

Date	Culture	Method of Exposure	Results of Exposure (Hours)	
			Growth	No Growth
September 7, 1905.....	No. 101	On egg medium	2, 5, 9½, 15, 22½, 30	.....
" 7, 1905.....	" 101	On paper+moisture	2, 5, 9½, 15, 22½, 30	.....
October 21, 1905.....	" 101	On egg medium	25, 30, 35, 40, 45, 50	.....
" 21, 1905.....	" 101	On paper	.....	25, 30, 35, 40, 45, 50
" 21, 1905.....	" 101	On paper+moisture	25	30, 35, 40, 45, 50
" 21, 1905.....	" 110	On paper	25	30, 35, 40, 45, 50

on paper slips show that diffuse light does have an effect; this is not due to desiccation, for when moisture was added the result was similar.

Taking the results for tubercle as a whole, there is no good reason to ascribe to this organism any special powers in the way of resistance or lack of resistance to sunlight; in fact, it possesses about the same order of reistance as the other nonsporogenous pathogenic bacteria.

A number of experiments were carried out to determine which rays acted bactericidally upon *B. tuberculosis*. The method adopted was to employ the paper slip cultures under a single pane of red, green, or blue glass. These glass panes were found, however, not to give pure monochromatic light, for the red filtered through the blue, and the blue through the red, while both filtered through the green. Under these circumstances, Tubercle No. 101 gave the following results:

TABLE 19.

Trial	Date	Method of Exposure	Result of Exposure (Min.)	
			Growth	No Growth
(a).....	May 3	Under colorless glass	.....	5, 10, 15, 20, 30, 45
(b).....	" 3	" red "	5, 10, 15, 20	30, 45
(c).....	" 3	" green "	5, 10, 15, 20, 30	45
(d).....	" 3	" blue "	5	10, 15, 20, 30, 45
(e).....	" 17	" colorless "	5	10, 20, 30
(f).....	" 17	" red "	5, 10	30
(g).....	" 17	" green "	5, 10, 20, 30	.....
(h).....	" 17	" blue "	5, 10	20, 30

These experiments indicate that it is the violet end of the spectrum that is fatal for tubercle, just as for other forms.

Since the data which have been recorded in the foregoing tables are distributed throughout the paper, it appears wise to collect them in a single table for convenience of reference and comparison. The table is as follows:

TABLE 20.

SUMMARY OF RESULTS OF EXPOSING BACTERIA TO DIRECT SUNLIGHT

Date	Organism	Method of Exposure	Limits of Life (Min.)
March 20.....	*B. coli*	Direct	0 and 2
" 23.....	"	"	2 " 4
" 23.....	"	"	1 " 2
April 7.....	"	"	2 " 5
" 11.....	"	"	0 " 2
May 11.....	" (No. 547)	"	2 " 5
" 11.....	" (No. 711)	"	2 " 5
" 11.....	"	"	2 " 5
April 27.....	*B. typhosus*	"	5 " 10
May 10.....	"	"	0 " 2
" 15.....	"	"	2 " 5
April 22.....	*B. dysenteriae*	"	0 " 2
May 9.....	"	"	0 " 2
" 10.....	"	"	2 " 5
April 11.....	*S. cholerae-asiaticae*	"	0 " 2
" 27.....	"	"	0 " 2
May 19.....	"	"	2 " 6
March 31.....	*B. diphtheriae*	"	5 " 10
April 27.....	"	"	0 " 2
May 10.....	"	"	2 " 5
April 2.....	*M. pyog. aureus*	"	10 " 15
" 22.....	" "	"	5 " 10
May 15.....	" "	"	5 " 10
April 7.....	*M. tetragenus*	"	2 " 5
" 11.....	"	"	2 " 5
" 22.....	"	"	2 " 5
May 10.....	"	"	0 " 2
April 27.....	*B. prodigiosus*	"	0 " 2
May 11.....	"	"	2 " 5
April 7.....	*B. pyocyaneus*	"	0 " 2
" 11.....	"	"	2 " 5
May 9.....	"	"	0 " 2
June 30.....	"	"	2 " 4
April 2.....	*Sar. aurantica*	"	25 " ..
May 15.....	"	"	20 " ..
" 19.....	"	Under glass	60 " ..
" 28.....	"	Direct	60 " 90
" 15.....	Pink air-coccus	"	20 " ..
" 19.....	" "	Under glass	60 " ..
" 28.....	" "	Direct	90 " 120
" 12.....	Möller's grass bacillus	"	15 " ..
December 24...	Tubercle 101 (human)	Under glass	0 " 10
" 26...	"	" "	30 " 60
" 26...	"	" "	0 " 10
April 4.....	" 101	Direct	15 " 20
December 26...	" 102 (human)	Under glass	10 " 20
April 11.....	" 102 (human)	Direct	0 " 5
December 26...	" 110 (bovine)	Under glass	0 " 10
" 26...	" 113 (avian)	" "	10 " 20
April 23.....	" 113	Direct	15 " ..

PRACTICAL BEARINGS OF THE RESULTS.

In reading the publications of the workers in this field, especially those of the earlier investigators, one notices that they expected rather more disinfectant action from the sunlight than was found; and so there seems to be something of a tone of disappointment. However this may be, it is certain that the results they obtained fell far short of doing justice to the disinfecting action of the sun's rays. While this action has been considered important from a sanitary view-

point, it is certain that it is much more powerful than previous results have indicated. Were it not for this powerful repressive agent, coupled especially with the repressive action of desiccation, it seems, indeed, that our chances for waging a successful war against disease and other objectionable bacteria would be slight; but when we realize that 2 to 10 minutes of active sunlight are sufficient to kill them when directly exposed, we can readily understand how the vast majority of all such bacteria are effectively destroyed, and only an infinitesimal number remains. This gives sanitary science new hopes and fresh courage with prospects of the highest success.

These results explain, in a measure, the advantages of a dry climate, such as the western and southwestern portions of the United States possess, where, due to the dryness and the superabundant sunshine, most bacteria, and especially the non-spore-bearing disease germs are quickly destroyed. Above all, they emphasize the importance of well-lighted and ventilated houses. The sunlight is a friend and protector of our welfare and should not be barred from our homes by shutters and heavy shades; for there is truth in the Italian saying: "Where sunlight enters not, there the physician goes."

CONCLUSIONS.

The conclusions arrived at may then be summarized as follows: The methods heretofore employed in testing the bactericidal action of sunlight do not seem to be well suited for this determination, since the results do not indicate the full power of this agent.

The light is absorbed by the medium in which the bacteria are planted, and the glass cover both absorbs and reflects a considerable portion of the effective rays. A more suitable method consists in planting the bacteria upon glass or paper and exposing directly, i. e., without glass or other cover, to the sun's rays. By this method most of the non-spore-bearing bacteria, including *B. tuberculosis*, *B. diphtheriae*, *B. typhosus*, *S. cholerae-asiaticae*, *B. coli*, *B. prodigiosus*, and others, are killed in a remarkably short period of time, varying from 2 to 10 minutes. This time is considerably lengthened if the suspension used is not homogeneous and the bacteria consequently become clumped or bunched in the film. Certain saprophytic bac-

PLATE 2.

PLATE 3.

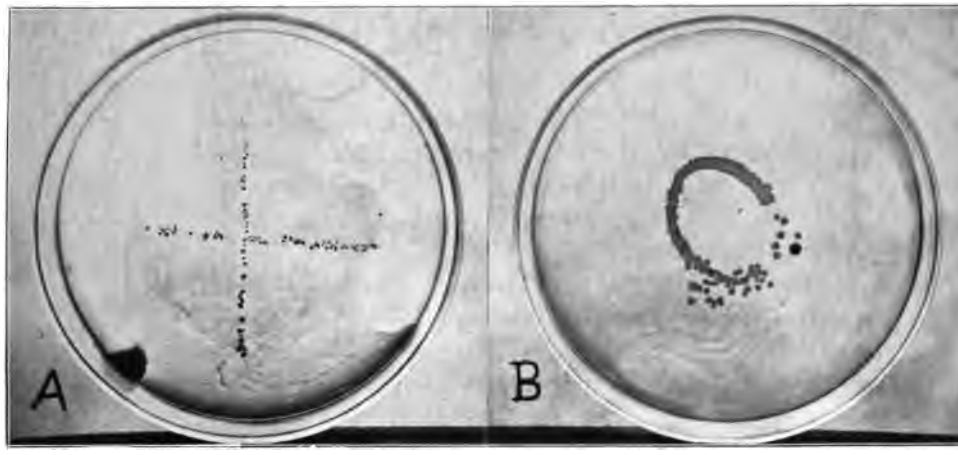


FIG. 1.

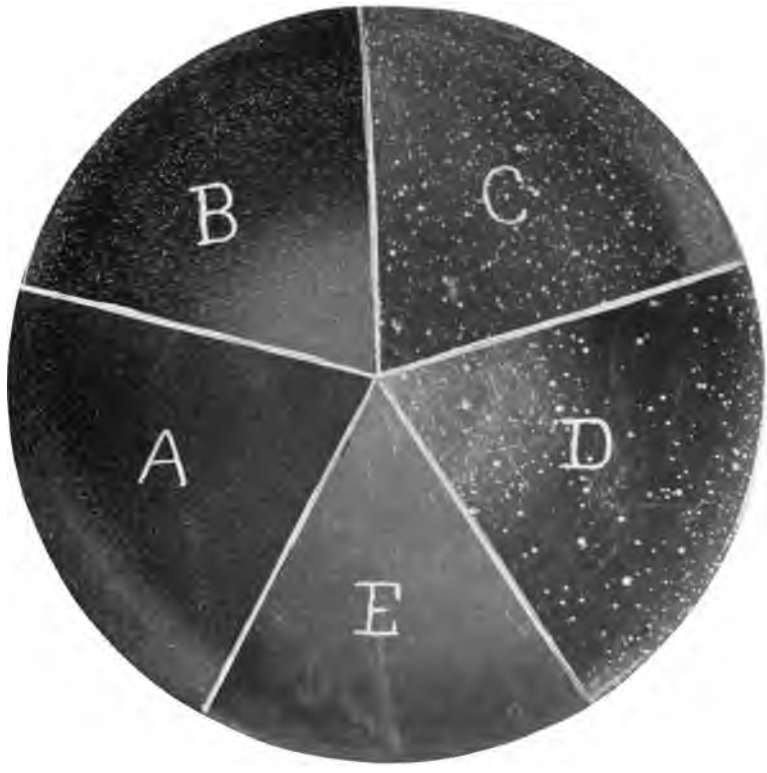


FIG. 2.

teria, such as the micrococci found in the air, require a much longer period of exposure to kill them, the time being upward of an hour.

By the method employed in the foregoing work, practically all the disturbing and complicating factors of the older methods are eliminated: the time required for exposure is so short that desiccation becomes a negligible factor, or practically so; the formation of bactericidal by-products in the medium, such as hydrogen peroxide, phenol, organic peroxides, etc., is entirely removed, and consequently the action must be ascribed wholly to the sunlight.

So far as *B. tuberculosis* is concerned, it is the more refrangible rays, or the violet end of the spectrum, which are bactericidal for this organism.

Finally, the results by direct exposure of the bacteria indicate that sunlight is a much more powerful germicidal agent, and consequently a more important hygienic factor, than it has heretofore been considered; that the bacteria, when freely exposed, are killed in one-fifth to one-twentieth of the time formerly considered necessary.

ACKNOWLEDGMENTS.

In closing I wish to express my deepest gratitude to Professor H. L. Russell for suggesting and directing the present investigation; I have also to acknowledge with thanks the kindly assistance of Professors W. D. Frost and E. G. Hastings, and Dr. C. A. Fuller, all of whom have been ever ready to promote and encourage the work.

EXPLANATION OF PLATES.

PLATE 2.

Tubercle cultures grown on egg medium after exposing to direct sunlight as follows: A—½ hr.; B—1 hr.; C—1½ hrs.; D—2 hrs. (no growth). Note the area of the infected paper in each tube.

PLATE 3.

FIG. 1.—Showing petri plates exposed uncovered to direct sunlight. A is *M. tetragenus* exposed 2 minutes; B is a pink micrococcus from the air, and was exposed 60 minutes. The cross and circle are made up of the bacterial colonies; they serve as mechanical devices for distinguishing the culture inoculated from the foreign or contaminating bacteria.

FIG. 2.—*B. coli* in agar plates exposed to sunlight. A = control; B = 10 min; C = 20 min.; D = 30 min.; E = 45 min.

SOME DATA IN REGARD TO THE COMPARATIVE DISPOSITION OF ORGANIC MATTER BY SAND, CONTACT AND SPRINKLING FILTERS.

H. W. CLARK.

The primary object in sewage purification is to change by bacterial oxidation the putrefying matter or matters easily subject to putrefaction present in sewage to a more stable form and thus convert an ill-smelling, objectionable liquid into one free from odor and easily decomposable matter. Only a small percentage of the total organic matters present are of this easily changeable nature, however, and it is the large remainder, both organic and mineral that accumulates in settling tanks that causes filter clogging and is the chief element of cost in adequate sewage disposal. It is well recognized to-day that in the successful treatment of sewage upon a large scale, the ultimate disposition of suspended matter presents the chief difficulty.

At the present time, three classes of sewage filters are well-known and have been studied at many places, namely, sand, contact and sprinkling or trickling filters, and the object of the present paper is to show the relative amounts of total matter, matter in suspension, etc., that reaches such filters when operated at usual or normal rates, and the disposition that each class of filter makes of this matter; and, as said before, the study is largely of the organic matter in suspension in sewage.

In average Massachusetts sewage from 50 to 75 per cent. of the matter in suspension is organic and only from 1 to 7 or 8 per cent. is nitrogen — rarely more than 2½ per cent.— this percentage varying with different sewages, and of course, at different times with the same sewage, etc. The remainder of the organic matter is carbonaceous, resistant to bacterial action and hence slow to putrefy or decompose. If we look at the filtration problem then from the point of view that successful purification is accomplished whenever the putrefying matters present in sewage are broken up, oxidized and rendered inoffensive, then the filter that will accomplish the greatest amount of this work upon the smallest area is the most successful filter. If, on the other hand, we consider successful filtration accomplished only when practically all the organic matter present in sewage, both stable and unstable, is removed, then a very

different problem is presented. Sand filters only can possibly fulfill the latter requirement, while contact and sprinkling filters can be made to fulfill the first requirement.

In studying the work in disposing of matter actually reaching them of each of the three classes of filters mentioned, I shall make comparisons taken from the work of sand, contact and sprinkling filters operated at the Lawrence Experiment Station. The comparisons are based upon the work and present condition of sand filters that have been operated 19 years, contact filters that have been operated 6 years, and sprinkling filters that have been operated nearly 8 years, each and all receiving Lawrence sewage.

The Lawrence sewage reaching the experiment station is a strong domestic sewage, much stronger than the average sewage of American municipalities now operating sewage purification plants and containing an amount of matter in suspension fully equal and comparable with its strength and other respects. The matter in suspension in this sewage is more or less finely divided, but not more so than the matter in many sewages that have had a slight preliminary screening, or sedimentation for the removal of coarser matters in suspension, or that have traveled through a considerable length of sewer before reaching the disposal area.

The total matter in the average station sewage of the last 19 years has amounted to about 2,800 pounds per million gallons, and the suspended matter to about 2,080 pounds per million gallons. The matter in suspension has had a composition about as follows: 70 per cent. organic or volatile matter and 30 per cent. mineral matter; of the organic matter about 2.6 per cent. is organic nitrogen, and the remainder carbonaceous matter, containing about 40 per cent. carbon, as shown by many analyses, and 22 per cent. fat or fatty matters.

SAND FILTERS.

For nearly twenty years experimental sand filters and municipal sand filters have been operated in Massachusetts. Experimental filters operated at an average rate of about 70,000 gallons per acre daily have given an effluent nearly always clear and much of the time practically colorless and with little or no odor. Municipal filters have been operated at average rates of from 40,000 to 60,000 gallons per acre daily with as good as or even better removal of organic matter than the experimental filters, and hence have pro-

duced effluents of fully as good and often of better quality than the experimental filters. To show by actual figures what intermittent sand filters accomplish in the disposal of organic matter, the average result of a Lawrence experimental sand filter, not the one that has given the purest effluent, but one that has given average results, can be taken. This filter has been operated at an average rate of 70,600 gallons per acre daily for 19 years and the average analysis of its effluent for its entire period of operation is as follows:

AVERAGE ANALYSIS OF THE EFFULGENT OF FILTERS NO. I.

(Parts per 100,000.)

Quantity Applied (Gals.)	Color.	Ammonia. Free.	Albuminoid.	Chlorine.	Nitrogen as Nitrates.	Nitrites.	Oxygen Consumed.
70,600	.36	.7492	.0674	8.45	2.28	.0197	.59

This effluent has been practically free from matters in suspension, probably 95 of every 100 samples collected showing none, and the other five containing so little that it would be lost in averaging. Hence it may be assumed that practically none of the organic or mineral matter in suspension in the applied sewage has appeared in this effluent.

There has been applied with each million gallons of sewage about 375 pounds of nitrogen, and of this, 18 per cent., or 67.5 pounds, has been organic nitrogen. Of the total nitrogen applied, including both that in solution as well as that in suspension, about 55 per cent. has been oxidized and has appeared in the effluent; about 18 per cent. has been present in the effluent unoxidized; about 3 per cent. has been stored in the filter and 25 per cent. has been liberated by nitrogen-liberating bacteria and passed into the air. Of the organic nitrogen applied, largely nitrogen of the suspended matters, about 65 per cent. has been oxidized, about 16 per cent. stored and about 19 per cent. has disappeared through the action of the nitrogen-liberating bacteria. The nitrogen in sewage, however, while of course forming an integral part of the organic matter that is liable to quick putrefaction, the production of bad odors, etc., and hence the portion that must be broken up by bacterial action, is but a small constituent of the total organic matter in sewage. Under the right conditions for filtration it can be easily and quickly disenchained from its associated elements and the putrefying bodies thus broken up. It is the non-putrefying, slowly decomposable organic matter that must be finally reckoned with, and especially the organic mat-

ter in suspension. For instance, there has been applied to Filter No. 1 in each million gallons of sewage about 2,800 pounds of organic matter judging from loss on ignition results, and of this total about 2,100 pounds has been organic matter in suspension in the sewage, largely carbonaceous matter, as before stated, as the organic nitrogen forms but 3.3 per cent. of the total weight. Taking into consideration the rate of filtration, there has been applied to this filter about 62,000 pounds per acre per year of organic matter, and of this 37,800 pounds has been organic matter in suspension in the sewage. There has accumulated in the filter about 10,500 pounds of organic matter per acre per year of service, and of this, only 2.5 per cent., or 262 pounds, is organic nitrogen. Of the total organic matter applied, 17 per cent. has accumulated in the filter, but as the accumulation has been, of course, of the organic matter in suspension, the true comparison is with this, and of the 37,800 pounds of organic matter in suspension applied per acre per year, the analytical results show a storage of 28 per cent. Of this stored matter, 65 per cent. is in the upper foot of filtering material, but there is not enough stored as yet to prevent good operation of the filter and the production of a highly nitrified effluent. This organic matter, on account of the long period of storage of most of it and the resulting working over by bacteria, has been changed materially; in the matters in suspension in the applied sewage the fats are about 35 per cent. as great as the loss on ignition, but in the stored matter the fats are only about 2.8 as great as the loss on ignition. The amount of nitrogen in the suspended matter in the sewage has been about 6 per cent. as great as the amount of carbon in this matter, while in the stored matter it is about 2.5 per cent. The results of the many years of operation of this typical sand filter can be summarized as follows: At an average rate of 70,000 gallons per acre daily, a rate requiring 14.3 acres per million gallons of sewage cared for per day, it has produced a clear, highly nitrified and generally odorless effluent, has successfully disposed of the nitrogen in solution in the applied sewage and 84 per cent. of the organic nitrogen. It has oxidized and successfully disposed of 72 per cent. of the total applied organic matter in suspension in the sewage and has stored 28 per cent. of this matter. The residuum of organic matter stored is of an almost undecomposable, stable nature and increases slowly but continually with continued use of the filter. It is probable, however, that the relative percentage

stored will decrease and the percentage oxidized will increase as the filter continues in operation. That is to say, if this filter continues in operation for another ten years, the per cent. of the matters applied up to that date that will have been stored will probably not be greater than 20 per cent., and the matters oxidized will have increased to 80 per cent., this being due to the fact that this comparatively stable matter does change slowly and shows some relative decrease.

CONTACT FILTERS.

Contact filtration has been studied at the Lawrence station for the past twelve years, and many contact filters have been operated. For discussion in this paper, I have selected three of these filters, namely, Nos. 175, 176 and 221.

Coke contact filters Nos. 175 and 176 have been in operation about five years, and contact filter No. 221, constructed of broken stone, about three years. The average rate of operation of coke Filter No. 175 has been about 621,000 gallons per acre daily, of coke Filter No. 176, 595,000 gallons per acre daily, and of broken stone Filter No. 221, 536,000 gallons per acre daily; these rates being from $7\frac{1}{2}$ to $8\frac{1}{2}$ times as great as those of the sand filter already discussed, or in other words, these filters can receive and have received in each year from $7\frac{1}{2}$ to $8\frac{1}{2}$ times as much sewage per unit area of surface as the sand filters. The average analysis of the effluent of each of these filters for their period of operation is shown in the following table:

AVERAGE ANALYSIS OF THE EFFLUENT OF FILTERS NOS. 175, 176 AND 221 DURING THEIR PERIOD OF OPERATION.

Filter No. 175.

(Parts per 100,000.)

Quantity Applied.	Temp. Deg. F.		Appearance.		Ammonia.				Nitrogen as		Oxygen Consumed.	Bacteria per Cubic Centimeter.
						Albuminoid.						
Gallons per Acre Daily for Six Days in a Week.	Sewage.	Effluent.	Turbidity.	Color.	Free.	Total.	In Solution.	Chlorine.	Nitrates.	Nitrites.		
620,800	57	62	6.7	.57	1.1290	.1887	.1302	11.16	1.97	.0307	1.32	475,400

Filter No. 176.

Quantity Applied.	Temp. Deg. F.		Appearance.		Ammonia.				Nitrogen as		Oxygen Consumed.	Bacteria per Cubic Centimeter.
Gallons per Acre Daily for Six Days in a Week.	Sewage.	Effluent.	Turbidity.	Color.	Free.	Albuminoid. Total.	Albuminoid. In Solution.	Albuminoid. Chlorine.	Nitrates.	Nitrites.		
594,700	57	61	8.8	.68	1.5384	.2065	.1455	10.57	0.91	.0195	1.42	465,100

Filter No. 221.

Quantity Applied.	Temp. Deg. F.		Appearance.		Ammonia.				Nitrogen as		Oxygen Consumed.	Bacteria per Cubic Centimeter.
Gallons per Acre Daily for Six Days in a Week.	Sewage.	Effluent.	Turbidity.	Color.	Free.	Albuminoid. Total.	Albuminoid. In Solution.	Albuminoid. Chlorine.	Nitrates.	Nitrites.		
536,300	56	56	9.0	.94	2.1921	.2888	.2055	10.79	0.16	.0069	2.30	715,200

There has been applied to coke Filter No. 175 with each million gallons of sewage about 2,130 pounds of organic matter, this filter having received strained sewage; to Filter No. 176, 2,770 pounds, and to stone Filter No. 221, 2,810 pounds with each million gallons of sewage; or in other words, taking into consideration the rate of filtration of each, 400,000 pounds per acre per year to coke Filter No. 175; 494,000 pounds to coke Filter No. 176, and 450,000 pounds per acre per year to stone Filter No. 221, or from 6 to 8 times as much per acre per year to these filters as to the sand filter previously discussed. Each million gallons of sewage applied to coke Filter No. 175 has contained on an average 800 pounds of suspended matter; each million gallons applied to Filter No. 176, 1,760 pounds, and each million gallons applied to Filter No. 221, 1,710 pounds of suspended matter, or 149,000, 314,000 and 278,000 pounds per acre per year to Filters Nos. 175, 176 and 221, respectively. Of the suspended matter practically 75 per cent. has been organic, and coke Filter No. 175 has received 112,000 pounds of organic

matter in suspension per acre per year; Filter No. 176, 236,000 pounds, and Filter No. 221, 206,000 pounds. Filter No. 175 has received with each million gallons of sewage about 350 pounds of nitrogen, and of this about 23 per cent., or 70 pounds, has been organic nitrogen. The corresponding figures for Filters Nos. 176 and 221 are 390 and 78 pounds, and 380 and 114 pounds, respectively. Of the total nitrogen applied to Filters Nos. 175, 176 and 221 there has appeared oxidized in the effluent of each 49, 19 and 4 per cent., respectively; 2, 1 and 10 per cent. of the total applied nitrogen has been stored and 18, 46 and 37 per cent. for each filter, respectively, liberated. Of the organic nitrogen applied to Filters Nos. 175 and 176, 6 per cent. has been stored, and Filter No. 221 has stored 32 per cent. of the organic nitrogen applied. Of the total organic matter applied, Filter No. 175 has stored 5 per cent., Filter No. 176, 6 per cent., and Filter No. 221, 26 per cent. Of the suspended solids in the sewage, they have stored 13, 8 and 36 per cent., respectively, and of the organic matter in suspension applied, they have stored 17, 10 and 47 per cent., respectively, or relatively, 60, 3.6 and 168 per cent. as much as the sand filter already discussed. Of solids in suspension, Filter No. 175 has stored 19,400 pounds per acre per year; Filter No. 176, 25,000 pounds per acre per year, and Filter No. 221, 97,000 pounds per acre per year. In other words, they have stored 100, 135 and 610 pounds, respectively, of suspended matter for each million gallons of sewage applied, and of this stored matter 45 per cent. is organic. These filters have produced effluents of varying character: Filters No. 175 and 176, effluents with much of the easily putrescible matter of the sewage oxidized or removed, and hence, generally, stable effluents notwithstanding the large amount of organic matter present, while Filter No. 221 has produced much of the time an effluent subject to putrefaction. These filters require about 1.7 acres of surface per million gallons of sewage per day.

SPRINKLING FILTERS.

For the third comparison of the disposition of matter by filtration, the results of two sprinkling filters that have been in operation eight years are presented. These two filters are constructed of broken stone, and have received sewage at an average rate of 1,450,000 gallons per acre daily. During some of the years of their operation they have received raw sewage, and during other years,

sewage from which a portion of the matters in suspension have been removed, either by septic tanks or mechanical strainers. Averaging the results of these two filters, the following data are obtained: With each million gallons of sewage there has been applied 2,300 pounds of total organic matter and 875 pounds of organic matter in suspension, or about 380,000 pounds of organic matter in suspension in the sewage applied to each filter per acre per year. The filters have stored 2 per cent. of the organic solids applied, or 23,000 pounds per acre per year, 4 per cent. of the total suspended solids applied, and about 5½ per cent. of the organic matter in suspension in the applied sewage, 21,000 pounds per acre per year, or about 50 pounds per million gallons of sewage applied. There has been applied with each million gallons of sewage 375 pounds of nitrogen, and of this, about 17 per cent., or 64 pounds, has been organic nitrogen. To each filter there has been applied 28,000 pounds of organic nitrogen per acre per year, this organic nitrogen being about 7½ per cent. of the applied suspended solid matters. The filters have stored 1 per cent. of the total nitrogen applied and 6 per cent. of the organic nitrogen; 42 per cent. of the applied nitrogen has appeared oxidized in the effluent, 43 per cent. has appeared unoxidized, 14 per cent. has been liberated, and 1 per cent. has been stored.

SUMMARY.

All these results show clearly the true function and action of the three classes of filters in handling the large amount of fairly stable matter, organic and mineral, that reaches them. Oxidation of the matter actually putrefying at the time of application of the sewage is generally easily accomplished by any properly operated filters. This being done, a good sand filter, owing to oxidation and straining, produces a clear, odorless and highly-nitrified effluent. The rate at which it can be operated is limited, however, and can rarely exceed 100,000 gallons per acre daily when receiving a normally strong sewage. Preliminary treatments of the sewage, of course, allow this rate to be materially increased. Contact filters allow rates six to eight times as great as sand filters, but only the best give non-putrescible effluents, and trickling filters can be operated with normal sewage at rates at least three or four times as great as contact filters and twenty times as great as sand filters, with the production of a highly-nitrified, practically odorless and

non-putrescible effluent,—generally a quite turbid effluent, however. Sand filters, owing to their comparatively low rate of operation, are many years in accumulating enough stable organic matter to necessitate removal or cause difficulty in operation. A considerable percentage of that applied does, however, accumulate. The best of contact filters, on the other hand, and good trickling filters, succeed in oxidizing the putrefying matter present in the sewage as applied to them and owing to the nature of the materials of which they are constructed, accumulation is slow, as they pass along most of the non-putrefying, stable organic and mineral matters of the applied sewage. The percentage of those matters stored by trickling filters at Lawrence is slight, and apparently *does not gain* beyond a certain point in the best filters, as shown by our analyses and examinations. The peeling of this matter from the filtering materials at times of bacterial activity and its passage in large quantities into the effluent has been frequently noted.

In conclusion, then, this paper is simply a collection of data to show with some degree of accuracy what the final disposition of organic matter by various sewage filters really is. We no longer imagine that we can by bacterial action in properly constructed filter beds dispose of all organic matter. Much of it is too stable for such disposition; it will accumulate. In sand filters its accumulation is slow simply because the rate of filtration is necessarily low. There is much talk at the present time of higher rates of filtration through sand filters, and if poorer purification can be allowed and frequent sand removal resorted to economically, these higher rates are allowable. Sprinkling filters and the best contact filters give a less degree of purification, estimated by the matter oxidized or matter strained out, but allow the greater rates shown here, oxidize the putrefying matters and pass along a large percentage of the stable matter.

DATA SHOWING WORK OF SAND, SPRINKLING AND CONTACT FILTERS.

	Filter No. 1.	Filter No. 135.	Filter No. 136.	Filter No. 175.	Filter No. 176.	Filter No. 221.
Percent. Stored in Filter of.						
Total Nitrogen Applied	3	1	1	2	1	10
Organic Nitrogen Applied	16	6	6	6	6	32
Organic Solids Applied	28	2	2	5	6	26
Solids in Suspension Applied	.....	4	4	13	8	36
Per Cent. of Organic Matter in Solids in Suspension Applied	70	74	74	73	75	75
Per Cent. of Organic Nitrogen in Solids in Suspension Applied	3.3	6	9	6	5	5
Per Cent. of Organic Nitrogen in Stored Matter	2.5	8	8	3	6	6
Per Cent. of Organic Matter in Stored Matter	.....	55	60	45	46	43
Per Cent. which Stored Organic Matter is of the Suspended Organic Matter in the Applied Sewage	28	5	6	17	10	47
Pounds of Organic Matter in Suspension Applied per acre per year	37,800	380,000		112,000	236,000	206,000
Pounds of Organic Matter stored per acre per year	10,500	23,000		19,000	25,000	97,000
Pounds of Organic Matter Stored per Million Gallons of Sewage Applied	477	48	50	100	135	610
Per Cent. of Applied Nitrogen in Effluent, Oxidized	55	37	47	49	19	4
Per Cent. of Applied Nitrogen in Effluent, Unoxidized	18	39	47	31	34	49
Per Cent. of Applied Nitrogen Stored in Filter	3	1	1	2	1	10
Per Cent. of Applied Nitrogen Liberated*	25	23	5	18	46	37

*By difference.

DISSOLVED OXYGEN IN WATER.

SOME PRELIMINARY WORK ON A COLORIMETRIC PROCESS.

GILBERT H. PRATT and HERMAN STABLER.

In the course of a cooperative investigation, by the Rhode Island State Board of Health and the United States Geological Survey, of the pollution of streams, the writers have had occasion to make a large number of dissolved oxygen tests. We chose for this work the Winkler method which in brief is: First, the addition to the water of manganous sulphate and potassium iodide in sodium hydrate; second, the solution of the precipitate and liberation of iodine in proportion to the free oxygen in the water by the addition of sulphuric acid; and third, the titration of the resulting liquor by standardized thiosulphate with starch indicator.

This method requires either titration in the field with the attendant transportation of fragile apparatus or shipment of samples to the laboratory after the addition of reagents. It occurred to us that these objectionable features might be eliminated by use of a permanent standard on which the color of the liberated iodine could be read, since the free iodine is proportional to the dissolved oxygen in the water under examination.

Permanent standards for color, iron and ammonia having been used with much success it was thought desirable to use, if possible, one of these for our purpose, thus doing away with the necessity of preparing some new solution with a formula as yet undetermined. We found the platinum-cobalt color standard eminently suited for matching the iodine but it was at once apparent that greater depths of color would be required than for ordinary color work, a color standard of about 2800 being necessary for comparison with the highest colors of the dissolved oxygen test. For our preliminary work we adopted an arbitrary standard which was 100 millimeters depth of platinum-cobalt standard solution having a color of 500. After bringing samples of water in the usual way, up to the point of titration, we determined the depth required to match our standard, and also found the dissolved oxygen content, by titration. Furthermore, series of determinations were made upon solutions containing known amounts of oxygen as liberated from potassium permanganate. We thus found that the depth of solution required

to match our standard was inversely proportional to the amount of oxygen present and a factor was developed which, when divided by our depth reading in millimeters, gave dissolved oxygen in parts per million. Sufficient experimentation has not been done to establish this factor beyond question but our results indicate that 250 is its approximate value. Assuming this value as correct, by calculation we would infer that a reading in terms of color scale multiplied by .005 would give parts per million dissolved oxygen. While this inference is borne out in a general way by our further experimentation the value should not be taken as an absolute one but would do for field work where an accuracy of about .5 parts per million dissolved oxygen was sufficient, or for any investigations in which comparative values are desired.

In reading the color of the iodine on diluted portions, our work seems to indicate that dilutions of more than 1 to 10 should be avoided as the results on greater dilutions tend to run low using the formula color x.005 = parts per million. It also seems best to make readings on as high colors as convenient, or in other words, to make as little dilution as will bring the colors within the standards used, which for this work may easily be of greater intensity than for ordinary color work.

As a further perfection of the method for field work we have adapted it for use with the United States Geological Survey field outfit for color. Numerous readings made with the short tube (50 millimeters) on straight samples and on portions diluted as high as 1 in 8 gave very satisfactory results, using the factor previously given. We believe, however, that a few color discs higher than those now prepared for this method will be advantageous, a greater error being probable in field dilutions than in matching the more intense colors. While enough work along this line has been done to show us its feasibility, delay in the delivery of the higher color discs has prevented us from accumulating data as to the limits of accuracy of the method when applied in this way, which data we should have been glad to present with these notes. We can therefore merely state that our results indicate a probable error of approximately .5 parts per million using the factor previously given, an error that will be inappreciable in most dissolved oxygen work. Some of our work tends to show that the formula color x.005 = parts per million dissolved oxygen will give accurate results for waters with a medium content of free oxygen, low results for waters high in oxygen, and

high results for waters of small oxygen content. This has suggested to us the possibility that further work may develop a formula of the type (color x A) — B = parts per million dissolved oxygen which shall be equally applicable to determinations upon waters of high and low oxygen content.

We have found, also, that hydrochloric, nitric, or sulphuric acid can be used with equal accuracy for this method, if the acids are oxidized to a faint permanent pink with potassium permanganate, although nitric acid on account of its tendency to break up into oxides is open to suspicion after long standing. Either nitric or sulphuric acid can be used in the aluminum tubes provided with the United States Geological Survey color outfit.

In practice we have found that natural color and turbidity, unless excessive, do not seem to introduce an error great enough to require a correction. In extreme cases a color reading of the water after addition of acid, when computed to dissolved oxygen, may be applied as a subtractive correction.

In our work the only instance in which our colorimetric method failed was on a stream carrying starch in solution, in which case a blue color was of course developed upon addition of acid in the method routine.

It is to be regretted that more definite data cannot be put forward at this time, but the writers feel that they have obtained results of practical value which warrant their presentation to the Association.

OBSERVED RELATIONS BETWEEN DISSOLVED OXYGEN, CARBONIC ACID AND ALGAE GROWTHS IN WEEQUAHIC LAKE, NEWARK, N. J.

HERBERT B. BALDWIN, Newark, N. J., and GEORGE C. WHIPPLE, New York City.

1906.

The relations between carbonic acid and dissolved oxygen in water and the growth of microscopic organisms, such as the algae and protozoa, are well known in a general way, but few observations showing how direct this relationship is have been published.

The writers recently had occasion to make some observations bearing on this subject and a brief account of this is here presented. The occasion was that of a sudden destruction of fish which took place in August, 1906, in Weequahic Lake, Newark, N. J. As this destruction of fish is itself a matter of some interest an account of it is given by way of introduction.

DESCRIPTION OF WEEQUAHIC LAKE.

The lake in the Weequahic reservation of the Newark park system is really an artificial reservoir. Its construction was begun seven years ago when a roadway was built across Bound Creek. Three years ago this roadway was formed into a substantial dam.

The lake has a drainage area of approximately one square mile. Its water surface is about 80 acres. Its average depth is between five and six feet, but it is said that there are certain places in the lake along the old bed of the creek where the water is ten or twelve feet deep. The site of the reservoir before construction was a swamp, in which the mud and peaty material varied in depth from two to ten feet. Nearly half of its area was covered with a heavy growth of cat-tails. The peat and mud were not removed from the reservoir bottom at the time when the dam was built, but since then masses of peat, alder roots, old stumps, and cat-tails have been taken out as they have floated to the surface. Although great quantities of such matter have been removed, much remains. At the time of our inspection on August 21st we found many floating alder roots and masses of peat at the south end of the lake.

It is said that there are numerous springs at the bottom of the lake. Measurements have been made of the run-off which have shown that the average discharge is about 1.5 million gallons per day, — a high figure for a watershed of only one square mile, and indicating a considerable influx of ground water.

There are two inflowing brooks, shown on the accompanying map. One of these, a small one, flows through a cemetery; the other flows through a bog hole, entering the lake through a culvert under the railroad.

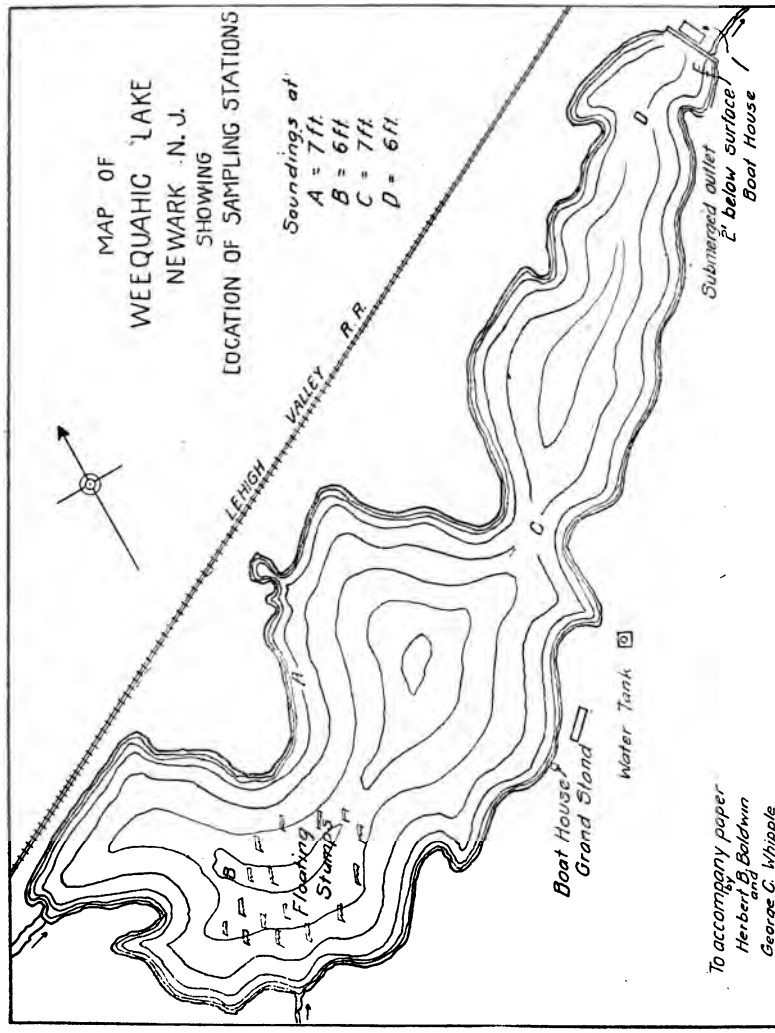
The bottom of the lake at the present time is covered with mud. Aquatic plants, water weeds and filamentous algae are conspicuously absent. It is said that during the last two years floating microscopic algae (the so-called plankton) have been present in large numbers during the summer, — in other words, the lake has shown the phenomenon of purging, or blooming. The algae growths have generally started in July and continued well into September. During August of the present year the algae have been much more abundant than usual.

The outlet of the lake is at the north end. Until recently there was a spillway five feet wide over the dam, but a few weeks before the death of the fish this spillway was closed and an outlet provided through a pipe passing through the dam. The intake of this pipe is less than two feet below the surface of the water.

DEATH OF THE FISH.

Sometime during Sunday night, August 19th, large numbers of fish died in the lake. When the attendants left the reservation on Sunday afternoon no dead fish were noticed around the shores, but on Monday morning they were found in great quantities. They continued to die during the day and also during Monday night. Twelve 2-horse loads of dead fish were picked up on Monday and buried, and it has been estimated that in all upwards of 120,000 fish, or more than fifteen tons, died during these two days. The dead fish included bass, roach (white fish), sun-fish, cat-fish (horn-pout), suckers, eels, and a few carp. They varied in size from sun-fish only two inches long to black bass weighing from five to six pounds, while one carp weighed nearly twenty pounds.

The dead fish were found in all parts of the lake. On Monday it was noticed that they were especially numerous near the two


MAP OF
WEEQUAHIC LAKE
NEWARK N. J.
SHOWING
LOCATION OF SAMPLING STATIONS
Soundings at
A = 7ft.
B = 6ft.
C = 7ft.
D = 6ft.
LEHIGH VALLEY R.R.
Floating Stumps
Boat House
Grand Slond
Water Tank
Submerged outlet
2' below surface
Boat House
To accompany paper
by
Herbert B. Baldwin
and
George C. Whipple

inflowing brooks. Instinct had apparently led them to seek the water entering the lake. Some of the eels even left the lake and traveled up one of the brooks for a considerable distance. Near the center of the lake there is a spring, and it was said that fish in the vicinity of this spring were found to be living and were more lively than some of the living fish found elsewhere.

The dead fish were removed from the lake with scoops as fast as they floated to the shore and were buried, with the intention of ultimately using them as a fertilizer.

CAUSE OF THE DEATH OF THE FISH.

It was thought by some of the people of Newark that the lake water was poisoned sometime during Sunday night. Others have thought that the lake had been dynamited. There seems to be no evidence to support either of these ideas. In fact, the available evidence is quite to the contrary. The lake is so large that its waters could not have been poisoned to an extent sufficient to kill the fish without using large quantities of the poison, or without having it thoroughly distributed through the general body of the lake. It does not seem likely that this could have been done. Examination of the dead fish did not show any abnormal appearance which could be attributed to poisoning. Neither did the fish bear any evidence of the lake's having been dynamited; no effects of concussion could be observed in the bodies of the dead fish. The fish did not show any evidences of having died from disease: no special organs of the fish were in an abnormal condition, and no evidences of fungus of bacterial growths could be observed about them other than those causing natural decay after death.

It does not seem to be necessary to resort to any of these theories to explain the cause of the death of the fish. A sufficient cause is found in the sudden exhaustion of oxygen in the water of the lake which occurred on Sunday, August 19th, and which was probably due to the decay of the algae. The fish apparently died by suffocation. On Monday it was noticed that many of the fish were gulping air at the surface of the water, and it was observed that very many of the fish died with their mouths open, — a phenomenon which has been observed before when fish have died from this cause. Examination of specimens of fish by Mr. C. H. Townsend, Director of the New York Aquarium, showed that they were

in a normal condition, as would be the case if they died from lack of air. The fact that many fish sought the incoming waters, which were well aerated, is also an evidence that oxygen was lacking in the water of the lake. Among the fish which were found dead a very large proportion were roach, and it is known that this fish is particularly susceptible to the exhaustion of oxygen.

EXHAUSTION OF OXYGEN FROM THE WATER.

We did not begin our observations until Tuesday, August 21st, after the dying of the fish had practically ceased. That the water was devoid of oxygen on Sunday and Monday seems a natural inference from the analyses which were made on Tuesday about 5:00 P. M. Samples of water were collected at different depths at both ends of the lake and tested in the field for dissolved oxygen. Near the south end of the lake it was found that at the surface the water was only half saturated with oxygen. At a depth of one foot the amount was only one-third of that required to saturate it, while below that depth the oxygen was practically absent. At the north end of the lake the surface water contained 79 per cent. of oxygen required to saturate it, while at a depth of three feet it contained 3 per cent. In other words, below a depth of one or two feet the water of the lake was practically devoid of oxygen.

That the exhaustion of oxygen was due largely to the decay of algae seems certain. During Sunday it was remarked by the attendants at the park that the color of the water was changing from green to a yellowish brown, and that as night came on the water became clearer than it had been. Our experience with algae growths in the past leads us to believe that these changes were due to the death of the algae and their settlement to the bottom of the lake. At the bottom of the lake the algae decomposed, as was evidenced by innumerable bubbles of gas which were seen rising through the water on Monday and Tuesday. This phenomenon was noticed by the men on Monday when the dead fish were first seen, and it was still strongly marked on Tuesday afternoon when we made our first examination. It seems likely that decomposition of the muck at the bottom of the lake also contributed to the exhaustion of oxygen. Furthermore, the oxygen being exhausted at the bottom allowed putrefaction to set in, the conditions being somewhat analogous to what goes on in a septic tank of

a sewage disposal system. Decomposition occurred to such an extent at the south end of the lake that great masses of roots and peat floated to the surface and drifted around as miniature islands. On disturbing this with a pole large volumes of gas were liberated.

The exhaustion of oxygen, then, may be said to have been due primarily to the decay of the algae, but partly to the decomposition of the peaty matter at the bottom of the lake.

EXAMINATION OF WATER FROM WEEQUAHIC LAKE.

Sample representing mixture of portions from different parts of the lake.

Date of Collection.	Date of Analysis.	Turbidity.	Color.	Nitrogen as					
				Albuminoid Ammonia.			Free Ammonia.	Nitrites.	Nitrates.
				In Solution.	In Suspension.	Total.			
Aug. 31, 1906....	Aug. 31, 1906....	100.	80.	.235	.870	1.105	.143	.000	.00

Oxygen Consumed.	Total Residue.	Loss on Ignition.	Fixed Residue.	Suspended solids.	Total Hardness.	Alkalinity.	Incrustants.
*7.4	210.	75.	135.	35.	102.	53.	49

* 4.2 in solution.

NOTE — The above analysis was made by Mr. H. B. Baldwin.

Microscopical analyses of the water made on Tuesday, August 21st, showed that the prevailing species present were Clathrocystis and Anabaena. The samples contained great masses of broken

EEQUAHIC LAKE AT VARIOUS POINTS,

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Date.	Depth in Feet.	STATION D. Temperature Fahrenheit.	Dissolved Oxygen. Parts per Million	Dissolved Oxygen. Per cent of Saturation.	Carbonic Acid. Parts per Million.	Date.	STATION E. Depth in Feet.	Temperature Fahrenheit.	Dissolved Oxygen. Parts per Million.	Dissolved Oxygen. Per cent of Saturation.	Carbonic Acid. Parts per Million.
Aug. 21.....	.....	82	.....	50	.....		.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....
	.....	.....	.....	3	.....		.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....
Aug. 22.....	.....	80	144	179	.....		.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....
	.....	.....	10.0	122	.....		.....	.....	.....	.....	.....
	.5	.....	0.6	8	.....		.....	.....	.....	.....	.....
Aug. 24.....	0	76	23	27	.....		.....	.....	.....	.....	.....
	.....	.....	2.5	29	.....		.....	.....	.....	.....	.....
	.....	.....	2.2	26	.....		.....	.....	.....	.....	.....
	3	.....	1.6	19	.....		.....	.....	.....	.....	.....
	5	.....	1.1	12	.....		.....	.....	.....	.....	.....
	6.5	.....	.....	.....	.....		.....	.....	.....	.....	.....
Aug. 27.....	0	74	0.6	6.5	.....		.....	.....	.....	.....	.....
	.....	76	9.0	109	3		.....	.....	.....	.....	.....
	.....	.....	8.8	104	.....		.....	.....	.....	.....	.....
	.....	75.5	8.0	94	5		.....	.....	.....	.....	.....
Aug. 31.....	0	80	18.0	225	< 0	Aug. 31	0	80	.....	.....	.....
	3	.....	13.5	113	< 0		3	79	7.1	70	< 0
	5	.....	4.5	56	< 0		.....	.....	.....	.....	.....
	6	76.5	2.2	27	9		.....	.....	.....	.....	.....
	.....	76.0	.....	.....	.....		.....	.....	.....	.....	.....
	.....	73.0	10.0	115	.....		.....	.....	.....	.....	.....
	.....	81	25.0	318	—2.3		.....	.....	.....	.....	.....
	.....	74	.....	.....	5.		.....	.....	.....	.....	.....
	.....	79	15.6	189	.....		.....	.....	.....	.....	.....
	.5	73	.....	.....	.....		.....	.....	.....	.....	.....
	.....	73	.....	.....	....		.....	.....	.....	.....	.....
	.....	69	.....	.....	.....		.....	.....	.....	.....	.....
	.....	69	.....	.....	.....		.....	.....	.....	.....	.....
	.....	47	10.1	86	3.0		.....	.....	.....	.....	.....
	.....	47.5	102	87	3.0		.....	.....	.....	.....	.....
	.....	48	10.1	86	3.0		.....	.....	.....	.....	.....

NOTE — When

down algae which could not be identified. Unquestionably the algae growth prior to Sunday must have been very heavy.

MICROSCOPICAL ANALYSIS.

(STANDARD UNITS PER C.C.)

Weequahic Lake Date 1906.	Sta. B, South End. Aug. 21.	Sta. D, North End. Aug. 21.	Sta. B, Surface. Sept. 3.	Sta. B, Surface. Sept. 7.	Sta. B, 3' deep. Sept. 7.	Sta. D, Surface. Sept 7.	Sta. D, 3' deep. Sept. 7.	Sta. D, Surface. Sept. 26.	Sta. B and D, Surface. Nov. 7.
Asterionella	.....	.....	.....	.....	.....	.....	.....	.....	140
Melosira	.....	.....	3400	2700	3000	1800	2000	1100	830
Synedra	100	20	.....	.....	.....	.....	.....	.....	230
Cyclotilla	.....	.....	.....	800	2000	4500	4000	60	1600
Scenedesmus	.....	.....	840	900	2460	500	600	80	180
Raphidium	.....	.....	.....	720	600	500	300	.....	.....
Anabaena	2750	6200	3440	1960	1800	3800	2500	300	.....
Clathiocystis	12500	10000	860	1340	1500	2500	2000	500	450
Aphanizomenon	.....	.....	.....	.....	.....	.....	.....	.....	250
Cladathrix	190	60	.....	.....	.....	.....	.....	.....	.....
Trachelomonas	.....	.....	40	60	20	480	500	.....	.....
Cryptomonas	200	70	320	700	80	600	500	200	.....
Vorticella	120	.....	.....	.....	.....	.....	.....	.....	.....
Coleps	100	20	.....	.....	.....	.....	.....	.....	.....
Ciliata	240	80	.....	.....	.....	.....	.....	.....	.....
Rotifer	100	.....	.....	60	.....	.....	100	.....	.....
Cyclops	50	200	.....	.....	.....	.....	.....	.....	.....
Total Organisms.	16350	16650	9020	9640	11460	15680	13500	2240	.....
Amorphous Matter	*500	*400	.....	.....	.....	.....	.....	.....	.....

* Both samples contained many disintegrated organisms. It is probable that the above figures are too low.

SUSCEPTIBILITY OF FISH TO EXHAUSTION OF OXYGEN.

We have been unable to find satisfactory data showing the limits to which oxygen in water may be reduced without affecting the fish life. That water entirely devoid of oxygen will not support fish life is well known, and that slight reductions in the amount of oxygen below the point of saturation do not materially affect fish life is also known; but the absolute amount of oxygen necessary for the life of various kinds of fish is not known with certainty.

Some experiments have been made in this connection by those interested in methods of sewage disposal. The ability of fish to live in sewage effluents has been frequently used as a test of satisfactory

oxygenation. In Clowes' work on "Experimental Bacterial Treatment of London Sewage" it is stated that "sewage effluents that contained 38 per cent. of the amount of oxygen required for saturation were capable of sustaining the life of gold-fish indefinitely, while in these same effluents less hardy fish, such as roach, dace and perch, became sickly after living in the effluent for a week or so." In Dibdin's "Purification of Sewage and Water" reference is made to a prolonged series of experiments on the Thames river water, which showed that when the percentage of aeration fell below 50 per cent. there was little chance of fish thriving in the water, and that when it fell below 30 per cent. no fish could sustain life for any prolonged period.

In Dr. Bruno Hofer's "Handbuch der Fischkrankheiten" (Munich, 1904) pp. 156-160, there are given some figures based on experiments, the substance of which is as follows:

Salmonoid fishes die if the oxygen content of water falls to 1.5 c.c. per litre (this is equivalent to 24% of saturation at 20°C, or to 19% at 10°C.); cyprinoid fishes, such as carp, goldfish, roach, etc., succumb when the oxygen content falls to 0.5 c.c. per litre. Fishes cannot utilize the last remnants of oxygen present in the water. In winter when metabolism is reduced, salmonoids require 3 and 4 c.c. per litre (35 to 45% of saturation at 55°C). Experiments which have been made by the United States Bureau of Fisheries have shown that black bass and yellow perch died in fresh sewage within seventeen hours, the oxygen content being about 1 c.c. per litre, while the same sewage when artificially aerated was harmless.*

In a private communication from Mr. J. M. Smith, Acting Fish Commissioner, he stated that in experiments made with trout a high mortality was found in waters which had a saturation of about 35 per cent.

Instances are on record where fish have been killed in streams by the discharge of manufacturing wastes which had no poisonous character, but which were of such a nature as, by their decomposition, to temporarily exhaust the oxygen from the water.

Different kinds of fish require different amounts of oxygen. It is said that eels get along with less oxygen than many fish. It seems certain, however, from the information at hand, that when

(* For this information and for the reference to Hofer we are indebted to Geo. M. Bowers, Commissioner, Bureau of Fisheries, Washington, D. C.)

the point of saturation of water falls as low as 20 per cent. or 30 per cent. there is sure to be a high mortality among the common fish of our lakes and rivers.

CAUSE OF THE DEATH OF THE ALGAE.

In order to get back to the primary cause of the death of the fish it remains to be ascertained what caused the sudden decomposition of the algae on Sunday, August 19th. As no examinations were made at the time we can only speculate as to what this was. Unquestionably several factors worked together to produce the sudden death of the algae. Of these the meteorological conditions which existed for a number of days before the phenomenon took place must have been very important. The weather had been extremely warm and the temperature of the water must have become very high at the surface. On August 21st the temperature of the surface water was 82°. In all probability it had been higher on preceding days, as the weather had been hot and the humidity high enough to prevent evaporation from the surface. There had been but little wind, and for a number of days previous the surface of the lake had preserved a mirror-like calmness. Under these conditions the lake water became temporarily stratified, with the warmer water at the top. During such quiet weather algae such as we found present on Tuesday tend to rise to the surface and form a scum. Becoming concentrated near the surface the tendency would be for their food material to become exhausted, while the high temperature itself would be prejudicial to their growth.

Another fact worth mentioning was the almost complete absence of aquatic plants from the lake. No water-weeds or eel grass, or even filamentous algae were to be found anywhere along the shores. On the other hand, the result of the fish fatality showed that the lake must have been to a very considerable extent overstocked with animal life. In other words, there was no equilibrium between the animal and plant life in the lake. Speaking generally, animal life makes a heavy drain on the oxygen contents of a natural body of water; plant life tends to restore it. Certain kinds of algae, namely, those which contain chlorophyl, are powerful oxygenators. There is some reason to believe that the blue-green algae which have been abundant in the lake during the present season are not as vigorous oxygenators as those algae which have green

chlorophyl instead of the blue-green modification of it. At any rate it has been noticed for many years that oxygen has been deficient in reservoirs where those organisms have predominated.

Algae require carbonic acid. In such a lake as that in the Weequahic reservation this is derived very largely from the decay of carbonaceous material at the bottom. When the water is in a state of circulation this carbonic acid manufactured by the bacteria at the bottom of the lake is distributed through the water, furnishing food to the algae. Stratification would tend to cut off the supply of carbonic acid from those organisms which were at or near the surface. Some algae appear to have some power to seize carbonic acid from the bicarbonates in the water, but there is a limit to which this can be done. In the present case the amount of carbonic acid that can be derived from the bicarbonates in the water is not great and probably would not be great enough to support the immense growth of algae which was present on August 19th.

The idea that some unknown cause killed both the fish and the algae does not seem tenable. Unquestionably the decay of the algae preceded the destruction of the fish, and the sequence of events is such as to believe that the case was one of direct cause and effect in the manner described.

RETURN TO NORMAL CONDITIONS.

The return to normal conditions occurred even sooner than was anticipated. On Wednesday, August 22d, a breeze sprang up which ruffled the surface of the lake at one end considerably. This led to a restoration of the oygen in the upper layers of the water. Two days later the wind further increased and resulted in the more complete oxidation of the water, especially at the end of the lake towards which the wind was blowing. Furthermore, another factor came in to still further increase the amount of oxygen. Following the decay of Anabaena and Clathrocystis other organisms began to make their appearance in large numbers. Among these were diatoms such as Melosira and Cyclotella, chlorophyllaceous algae such as Scenedesmus and Raphidium, and the chlorophyllaceous protozoan Cryptomonas. These organisms contain chlorophyl and are strong oxygenators. They developed with such rapidity that the oxygen in the water was increased beyond its point of saturation.

This interesting phenomenon led us to extend our observations over a longer period of time.

SURCHARGING OF THE WATER WITH OXYGEN.

Before describing the phenomenon of surcharging of the water with oxygen observed in Weequahic lake it may not be out of place to state that the amount of oxygen which water will hold in solution in contact with atmospheric air depends upon its temperature, being nearly double as much in winter as it is in summer. For each temperature there is a certain amount of oxygen which will just saturate water. This is termed a saturation of 100 per cent. If an analysis shows the presence of a smaller or larger amount than this, it is common to express the result in terms of the percentage of saturation. For example, if the per cent. of saturation is given as 50, it means that the water contains only half as much oxygen as it is capable of holding; if the percentage is given as 150, it means that the water contains 50 per cent. more oxygen than that required to saturate it, or, in other words, it is supersaturated or surcharged. Percentages higher than 100 are frequently observed in nature. In some cases they are due to the pressure of free oxygen liberated by growing plants.

A surcharging of the water with oxygen was first noticed in Weequahic lake on August 22d. At Station B the water at the surface contained 149 per cent. of that required to saturate it, while the water at a depth of one foot contained 110 per cent. Below a depth of two feet oxygen was deficient, as shown by the figures given in the accompanying table. At Station D on the same date the surface water contained 179 per cent. of that required to saturate it, while below a depth of two feet the conditions were about the same as at the other end of the lake, the percentages of saturation being low. The temperature observations on this date pointed to a marked stratification of the water.

On August 24th the stratification of the water had almost disappeared. This was shown by the fact that there was but little difference in the temperature of the water at the top and bottom, and also by the fact that the differences between the oxygen figures at the top and bottom were less. It was noticed that wherever the temperature difference was considerable the difference in the oxygen contents was also great.

As a result of the mixture of the water on August 24th through the action of the wind, the surface water ceased to be surcharged, while the oxygen contents in the lower strata increased. These same conditions were observed on subsequent dates, and in general it was found to be true that whenever the water became stratified the surface layers became surcharged with oxygen, while after a mixing up of the water there was an increase in the amount of oxygen at the bottom, due to a distribution through the vertical of the excess oxygen from the surface.

Tests of the water for free carbonic acid showed that, in general, the amount varied inversely with the amount of oxygen present. On those dates when the water was stratified and when the surface water was saturated with oxygen, the amounts of carbonic acid were low at the surface and high at the bottom. Apparently during the stagnant periods the water in the lower layers acquired carbonic acid, while the water in the upper layers acquired oxygen. When the waters became mixed up, through the action of the wind, there was a consequential evening up of both the oxygen and carbonic acid throughout the vertical.

In those cases where the result of the carbonic acid was recorded as 0 in the table it was observed that the water acquired a pink color immediately after adding the phenolphthalein, indicating the presence of normal carbonates. In those samples which were collected on September 10th and 21st observations were made to determine how much acid it was necessary to add to render the water neutral to phenolphthalein. The results are recorded as negative quantities of carbonic acid and indicate the amount of carbonic acid abstracted from the bicarbonates in the water by the growing algae. This power to abstract carbonic acid from the bicarbonates has been noticed by several observers, but no special attention has ever been called to the phenomnon.

It was not thought possible that the atmosphere could be responsible for the superabundance of oxygen at the surface, inasmuch as this occurred at times when the water was stratified rather than when the surface of the lake was ruffled by the wind. It was noticed that with the return of the oxygen to the water, after the decay of the blue-green algae, other microscopic organisms increased in amount as already mentioned. As these organisms contain chlorophyl they were naturally looked to as the source of this excess of oxygen.

After making a number of experiments to conclusively prove the accuracy of the analytical work, an experiment was made to determine whether or not the excess of oxygen was present in the structure of or attached to the organisms themselves. Samples of the water for the oxygen test were collected in such a way that the organisms were removed in situ before the samples were drawn from the lake. This experiment gave a negative result, and indicated that the excess of oxygen was present in the water itself. Further experiments showed that on letting the sample stand or on shaking it this excess of oxygen was readily liberated and the amount reduced to the normal degree of saturation.

The methods of examination used in the analytical work were essentially those of the "Standard Methods of Water Analysis."

The temperature of the water at depths beneath the surface was ascertained by a simple apparatus as follows: A wide mouth bottle holding about one litre (a quart milk bottle was found convenient) was fitted with a three hole rubber stopper; into the central hole a thermometer was inserted and into one of the others a tube about 7 millimeters in diameter was pushed nearly to the bottom, the upper end of this tube being flared a little to receive a small rubber stopper to which a string was attached and which could be pulled out when the bottle reached the desired depth. The water would then enter through the large tube and the air escape through a small tube in the third hole of the stopper. A bag of lead shot of sufficient weight was put in the bottom of the bottle to sink it.

This apparatus worked admirably and held sufficient water to get reliable temperature readings in such a shallow lake. The dissolved oxygen and carbonic acid samples were taken in 250 cubic centimeter bottles lowered to the desired depth in a weighted can, and filled by means of a device similar to that used for the temperature samples, described above.

The determination of dissolved oxygen was made by Winkler's method with manganese sulphate, sodium hydrate and potassium iodide, the iodide being liberated in equivalent amounts and estimated with N/40 sodium thiosulphate.

The free carbonic acid was estimated by titrating with N/22 sodium carbonate, using phenolphthalein as an indicator. When the water was colored pink on the addition of phenolphthalein, showing the presence of normal carbonates, the result was recorded as 0 in the table. At times the amount of normal carbonates was

measured by titration with N/22 hydrochloric acid. The results were recorded in the table as negative values of carbonic acid. They may be translated into terms of normal carbonates by multiplying the figures by 2.2.

CONCLUSIONS.

The observations above described illustrate and help to explain a number of facts regarding the growth of algae, among which are the following:

The energy of the sun's rays penetrating a body of water diminishes rapidly as the depth increases. It is known that the bleaching of colored water in a reservoir is confined to a comparatively narrow stratum near the surface. It is known that the bactericidal influence of light in water does not extend to depths of more than a few feet. It is known that in the case of filamentous algae growing on the shore or bottom of a lake the limit of growth is comparatively near the surface. The observations made show that this is also true in the case of the floating microscopic organisms. Apparently the vital activities of the plants were greatest during calm weather when the organisms, by reason of their low specific gravity or because of attached bubbles of gas, rose to the surface and came within the influence of the sun's rays. On days when the wind kept the water in circulation the vital activities were apparently less, although the elaboration of carbon may have gone on to some extent.

These are interesting facts when taken in connection with what is generally known in regard to the relations between wind and algae growths. It is known for instance that in certain bodies of water wind storms frequently put a sudden check upon the growth of such algae as Anabaena. It is known that, generally speaking, the blue-green algae are less abundant in large bodies of water where there is constant circulation and vigorous wave action than in smaller bodies of water which are frequently in a quiescent state. It is known that in lakes which have an irregular shore line algae growths are more likely to develop in coves than they are in the main body of the lake. Diatom growths, on the other hand, are more likely to occur during periods when the water is in circulation than during periods of stagnation.

The action of the wind may be stated as follows. By mechanically stirring the waters it tends to cause the disintegration of

fragile organisms. It tends to prevent organisms of low specific gravity, such as the blue-green algae, from rising to the surface, thus preventing them from coming within the range of the sun's active rays. In the case of diatoms, however, which are heavy and which tend to settle in the water, it tends to prevent their sinking and to keep them near the top of the water, thus favoring their growth. By stirring up the water and constantly exposing different portions of it to the atmosphere it tends to reduce the amount of carbonic acid in the water, thus depriving the organisms of a part of their food, and to aerate the water and increase the amount of dissolved oxygen present. It tends to bring to the surface the products of the decomposition of organic matter at the bottom of the lake, as well as any spores of organisms that may be resting there. If the circulation is a gentle one it may increase the amount of carbonic acid in the water by distributing through the mass that which is found at the bottom, but if the circulation is violent it may tend to decrease the carbonic acid by losses into the atmosphere at the surface.

The observations illustrate another interesting fact which has received but scant attention, namely, the power of certain algae to abstract half-bound carbonic acid from the bicarbonates of the alkaline earths. Whether all algae have the power of doing this cannot be deduced from the observations. Previous studies made by one of us and by Mrs. Ellen H. Richards* have shown that the blue-green algae at least have power of doing this.

Surcharging of water with oxygen is interesting in connection with the movements of microscopic organisms. It has been recently shown by Mr. D. D. Jackson that the movements of diatoms are due to the liberation of gases. It is probable that many of the movements of other microscopic organisms may be explained in the same way. Certainly minute bubbles of oxygen attached to microscopic organisms must have much to do with their vertical distribution. They act as miniature balloons to float the organisms to the surface.

The writers believe that many of these matters are worthy of further research at the hands of sanitary biologists. The reason for the occurrence of algae in various reservoirs has never been satisfactorily explained. It is known that many factors are involved.

* Isabel F. Hyams and Ellen H. Richards. Notes on Oscillaria prolifica. Technology Quarterly, Vol. XV, No. 3, Sept. 1902.

Questions of temperature, of light, effect of soil stripping, etc., have been given careful attention, but the important relations between the algae and the two gases, carbonic acid and oxygen, have never received the attention they deserve. The writers hope that during the coming year this subject may be studied experimentally by those who have opportunities for doing so.

THE PATHOGENICITY OF STREPTOCOCCUS LACTICUS.

P. G HEINEMANN.

That streptococci frequently occur in milk has been shown by Escherich, Beck, Bergey, Lammeris and Harrevelt, v. Freudenreich, Hölling, Brüning, Savage, Müller, Kaiser and others. The identity of these streptococci with Streptococcus lacticus, Kruse (B. lactis acidi, Leichmann) was not recognized, however. The experiments described in this paper show that milk streptococci (Str. lacticus) may become pathogenic by repeated passage through animals and that the lesions produced are similar to those produced by Str. pyogenes from human affections. The rabbit was chosen for these experiments on account of its susceptibility to streptococcus infection. Five strains of Str. lacticus and three of Str. pyogenes were employed. One of the strains of Str. lacticus was obtained from Prof. Harding, of the N. Y. Agric. Exp Sta., Geneva, N. Y., two were isolated from Chicago milk and two from commercial "starters." These starters were found to contain Str. lacticus practically in pure culture. The three strains of Strep. pyogenes were obtained from cases of tonsilitis, peritonitis, and meningitis, respectively. Cultural and morphological studies of these streptococci proved that the pyogenic and milk streptococci were alike except that milk was coagulated somewhat more slowly by Str. pyogenes. After passage through rabbits the characteristics were not materially changed. Previous to inoculation the various strains were cultivated successively in three tubes of serum broth, and then two c. c. of the last culture injected subcutaneously, or in a few cases intravenously. The organisms were recovered from the lesions by the usual methods and cultivated in ordinary broth from which succeeding injections were made.

The lesions produced in rabbits were briefly as follows:

(1) One of the strains of Str. lacticus from milk was passed through ten rabbits successively by subcutaneous inoculation. From a slight induration at the point of inoculation in the first rabbit the severity of the lesions increased with each succeeding animal, until eventually large abscesses were formed. The pus was sometimes of a tough, viscous consistency, sometimes softer and semi-

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fluid. The fifth animal died in 29, the ninth in 16 and the tenth in 9 days. Post mortem examinations showed purulent exudates in the pleural, pericardial and peritoneal cavities and numerous pus foci under the skin. Streptococci were readily recovered from the pleural and peritoneal cavities, the heart's blood, kidney, spleen and subcutaneous pus.

An intravenous injection of a culture of the same organism caused the death of the rabbit in 25 days. The lesions were similar in nature to those described. From a broth culture prepared from the heart's blood a normal rabbit was injected and likewise a rabbit of the same size which had received a subcutaneous inoculation with the same organism subcutaneously, but to all appearances had recovered from the effects. The former died in 17 days, the latter in 6 days, the lesions also being similar to those described.

(2) A second strain of Str. lacticus from milk produced no local effect. The animal after several days commenced to carry the head bent towards the left side, and when touched would walk in a circle. When put on its back it regained its foothold only after strenuous efforts. The animal seemed to recover gradually, although considerably emaciated. Unexpectedly after about six weeks it died. Post mortem examination showed a large amount of whitish semi-fluid pus in the pleural cavity. No other lesions could be detected. Streptococci were recovered from the pus and the heart's blood. V. Lingelsheim has recorded similar observations produced by streptococci and ascribes the peculiar effect to some toxin produced by the streptococcus.

(3) The other three organisms produced effects resembling those described under No. 1 with but slight variations. The virulence of one of these strains from a commercial starter increased more rapidly than any of the preceding ones and also produced an erysipelas-like reddening of the skin at the point of inoculation in one of the rabbits. The eighth rabbit inoculated with one of these strains died on the tenth day, the seventh of the other strain on the ninth day. The fifth and last strain caused a similar nervous trouble as described above.

(4) The three varieties of Str. pyogenes produced the same effects on rabbits as Str. lacticus, except that death occurred in a shorter time. Str. pyogenes from tonsilitis caused the sixth rabbit to die on the seventh day. Str. pyogenes from meningitis killed the

fourth rabbit in seven days. Str. pyogenes from peritonitis caused the third rabbit to die in eight days. The lesions were identical in every respect with those brought about by Str. lacticus. By a fortunate coincidence the tenth rabbit inoculated with Str. lacticus from milk and the third rabbit inoculated with Str. pyogenes from peritonitis died at the same time, the former after nine, the latter after eight days. Post mortem examinations were made simultaneously. The pathological conditions in the two animals were strikingly alike. Pus pockets under the skin in various places, the usual pericarditis and the purulent exudate in the pleural cavity, revealed identical conditions in both cases. Streptococci were recovered in both cases from the affected parts.

CONCLUSIONS.

(1) Streptococcus lacticus is closely related to Str. pyogenes, not only morphologically and culturally, but also in pathogenic property.

(2) By repeated passages through rabbits Str. lacticus increases in virulence. After five or more passages Str. lacticus in subcutaneous injection was fatal in doses of 2 c. c. of 24 hour old cultures. The same amount injected intravenously proved fatal to the first animal.

(3) The lesions produced by Str. lacticus in rabbits are of the same kind and extent as those produced by Str. pyogenes obtained from pathological conditions in human beings.

THE EXCRETION OF BORIC ACID FROM THE HUMAN BODY.

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In the studies which I have inaugurated for the determination of the effect on health and digestion of various substances added to foods, the first substance investigated was boric acid and borax. The object of this investigation was to administer these bodies in small quantities to young men over a long period of time and determine the effect upon health, digestion and the various metabolic processes. Incidental to this investigation, the fate of the boric acid in the human body was studied. It was found that, as had been pointed out by other investigators, by far the larger quantity of the boric acid administered was excreted through the kidneys. There was a marked difference in the action upon the urine between the borax and boric acid. When boric acid was administered, the acidity of the urine was considerably increased. When borax was administered, the acidity was diminished, the reaction sometimes becoming amphoteric and even alkaline.

The first experimental work was divided into five series, twelve young men being observed during each of the series. The time of the series varied somewhat in length. The first series began on the sixteenth of December, 1902, and ended on the thirteenth of January, 1903. The second series began on the nineteenth of January, 1903, and ended on the twenty-first of February, 1903. The third series began on the nineteenth of February, 1903, and ended on the nineteenth of March, 1903. The fourth series began on the twentieth of March, 1903, and ended on the twenty-second of April, 1903. The fifth series began on the twenty-fourth of April, 1903, and ended on the twenty-ninth of June, 1903.

In the first series there were administered altogether 150 grams of boric acid, half of it as borax. There was recovered in the urine 124.58 grams, or an average percentage of recovery of 83.05. In the second series the average percentage of recovery was 82.85, the total amount administered being 98 grams and the amount recovered 81.19 grams. In the third series there were administered

(*Published in the Journal of Biological Chemistry, 1907, Vol. III, page 11.*)

132.90 grams and there were recovered 84.90 grams, an average of 63.88 per cent. In the fourth series there were administered 99.50 grams and recovered 82.55 grams, equivalent to 82.96 per cent. In the fifth series there were exhibited 127 grams and recovered in the urine 95.47 grams, an average of 75.17 per cent.

The total quantity of boric acid (and borax as boric acid) administered during the investigation was 607.40 grams, of which there were recovered in the urine 468.69 grams, the average percentage recovered being 77.16.

It having been stated by some investigators subsequent to the publication of these results that the amount of boric acid excreted in the urine as given was too small, a supplementary experimental determination was made with three young men over a period extending from the twenty-first of January, 1905, to the fourteenth of February, 1905. In each case after the cessation of the administration of the substance the tests of the urine were continued, until for several days, by the method employed, no determinable quantity of boric acid was noticed. The percentages of boric acid eliminated in the six cases were as follows:

In No. 1, 83.51; in No. 2, 77.84; in No. 3, 91.55; in No. 4, 87.75; in No. 5, 84.26; in No. 6, 77.38.

The Thompson method (1) employed here was the same as that employed in Bulletin 84, Part I, "Boric Acid and Borax." Calcium hydroxide was used throughout and the only departure from the regularly outlined method was in redissolving and precipitating more times in order to free the occluded boric acid from the precipitate. The volume of solution which was obtained was kept constant throughout.

The second method with a few modifications is that of Fendler (2) and consists essentially of concentrating a portion of the sample in the usual manner, acidifying and immersing the turmeric paper strips in it. After sufficient time has elapsed to allow the liquid to travel up the paper it is compared with strips which have been immersed in a solution whose exact content of boric acid is known.

It is seen that in three subjects of the series there was more than 80 per cent. recovered, while in three others of the series there was less than 80 per cent. recovered. During this investiga-

(1) Sutton's Volumetric Analysis, 8th ed., p. 98.
(2) Zeit. Unter. Nahr. and Genuss. 1906, 11-137.

tion experiments were also made to determine whether any of the boric acid administered assumed a volatile state and escaped through the expired air, or if any of it was found in the respiration. The well known tendency of boric acid to assume the volatile condition, especially when heated with methyl alcohol and some other bodies, led to the supposition that it might be reduced in the system to the form in which it would be volatilized in the expired air. To determine this point the expired air was forced continuously through a solution of lime water for three hours. The lime water was afterwards tested for boric acid with a negative result. It appears therefore that no appreciable quantity of boric acid escapes through the lungs. On the other hand it was found that very considerable portions of the boric acid are excreted through the perspiration.

From the above data it is seen that the average percentage of administered boric acid, excreted in the urine in this series is 83.71, which is considerably greater than that given for the first series of determinations. When the data are looked over, however, it is seen that this discrepancy is easily accounted for. In the first set of investigations there was one series where the proportion of boric acid recovered in the urine was only 63.88 per cent., while in the present series there was one instance where the proportion excreted was 91.55 per cent. If these abnormal results be omitted from each of the investigations, it is seen that they are practically the same. Thus in the first series of observations, made in 1903, the average percentage of borax excreted in the urine, excluding the one phenomenally low case, is 82.15, while in the observations made in 1905, excluding the one phenomenally high case, the average percentage excreted is 81.01.

These data show that the relative quantity of boric acid excreted varies with the individual case and may extend from 63 to 91 per cent. of the whole amount ingested. These data seem to establish very conclusively that the average percentage of administered boric acid which is excreted by the kidneys is a little more than 80 per cent.

In the spring of 1906 another series of determinations was made, extending from the 4th to the 22d of April. The object of this determination was to determine the quantity of boric acid secreted in the perspiration, and the examinations of the urine after the exhibition of a small quantity of borax were continued until no further test for boric acid could be distinguished. On the first

day of the observation the urine was examined carefully for boric acid. None was found by the usual method of determination and a trace only by Fendler's method. On the 5th of April one gram of boric acid was given and on the 6th of April, 2 grams.

The examinations by the usual method showed that after the 11th of April, five days after the administration of the last borax capsule, there was no longer a sufficient quantity of boric acid in the urine to be determined. On the contrary, by Fendler's method there was still estimated to be on the 22d of April two-thousandths of 1 per cent. The total percentage of the 3 grams administered which was excreted in the urine in this case was 65.9. This indicates that there was a detention of a very large proportion of the boric acid administered in the system, and that this detention was of somewhat indefinite duration, small quantities of boric acid being given off in the urine 16 days after its administration. A second subject, treated exactly in the same way, showed a percentage excretion of boric acid in the urine by the usual method of 71.3. There was still an appreciable quantity of boric acid in the urine by Fendler's method on the sixteenth day after the administration of the capsules. A third subject, treated in exactly the same way, showed an excretion of 82.96 per cent. of boric acid in the urine and an estimated amount of three-thousandths of 1 per cent. in the urine on the sixteenth day after administration by Fendler's method.

The average quantity of boric acid excreted in the urine in these three instances is 73.3, showing that when only a small quantity of boric acid is administered a very much smaller percentage of it is recovered in the urine than when the administraton is continued over a long period, thus indicating very plainly the tendency to accumulate the boric acid in the system. This fact is accentuated by the further observation that even after sixteen days an appreciable quantity of boric acid was still excreted in the urine.

EXCRETION OF BORIC ACID IN THE FECES.

Considerable quantities of boric acid, when administered as described in the preceding pages, are excreted in the feces, but the total quantity is so small, compared with that eliminated in the urine, as to be of little importance from a merely chemical point of view.

In the case of subject No. 1 in the series of April, 1906, 1.12 per cent. of the administered borax was recovered in the feces. In the case of No. 2, .78 per cent., and in the case of No. 3, .68 per cent. The average shows .86 per cent. of the borax administered excreted in the feces. This number must be regarded as a minimum percentage of excretion, since only 3 grams of borax were administered in these cases, altogether, and the observations continued until no appreciable quantities of boric acid were found in the feces. Traces, however, still remained after the above observations were concluded. It is evident, therefore, that a weighable portion of borax, administered as above described, is excreted in the feces, probably only about one per cent. of the total quantity, where only a small quantity is exhibited for a short time.

EXCRETION OF BORIC ACID IN THE PERSPIRATION.

It is evident from a general idea of the method of excreting boric acid that weighable portions of it should be found in the perspiration. This theoretical consideration was verified on several occasions during the progress of the work in the earlier periods.

In the spring of 1906 it was decided to determine if possible quantitatively the amount of boric acid excreted in the perspiration. This was the principal object of the series of the spring of 1906, with the three men above mentioned. The method of determination, while perhaps not capable of absolute exactness, is one which at least determines approximately the proportional amount of boric acid recovered. The three men above mentioned, after having taken 1 gram of borax on the 5th of April, and 2 grams on the 6th, one in the morning and one at noon, were conducted to the hot room of a Turkish bath in the afternoon on the 6th of April. Before entering they were carefully washed in distilled water and thoroughly dried with an extracted towel. They were then placed in a suit of thick woolen under-clothing which had been previously washed, steeped in distilled water and extracted in this way until no further extract was removed, and then dried. They remained in the Turkish bath at a temperature of from 130° to 135° for an hour and thirty minutes. The suit of underclothing was then removed and the men, standing in a basin, were thoroughly sponged with distilled water, the washings saved and evaporated, and the residue added to the extract from the woolen suit. After con-

centration, the quantity of boric acid recovered was determined. The quantity of boric acid recovered in the perspiration in the case of No. 1 was .0299 gram, equivalent to 1 per cent. of the whole amount exhibited. The quantity recovered in the case of No. 2 was .0311 gram, equivalent to 1.03 per cent. of the amount exhibited. The quantity recovered in the case of No. 3 was .0777 gram, equivalent to 2.59 per cent. of the quantity exhibited. In the case of No. 3 it should be observed that the washings in distilled water were accidentally lost, so that the amount represented as recovered was simply that derived from the woolen suit.

The average percentage of boric acid recovered, based upon the amount exhibited, is 1.54. It is fair to assume that the amount of perspiration during the hour and a half spent in the hot room of the Turkish bath is practically equivalent to that of 24 hours of ordinary temperature, so that roughly we may assume that the average percentage of the exhibited boric acid excreted in the perspiration of 24 hours would be 1.54.

No determination was made of the quantity of boric acid which could be secured on subsequent days, although it is evident that so long as it remains in the system and is excreted in traces in the feces and in appreciable quantities in the urine, traces would also be found in the perspiration.

The general conclusion derived from these experimental data is that the total quantity of boric acid excreted in the feces and perspiration is not much if any over 3 per cent. of that administered during the ordinary period of observation. It is evident, therefore, that even including these quantities with those which are excreted in the urine, not over 85 per cent. of the total amount of boric acid exhibited in these experiments is in the three excretions mentioned.

EXCRETION OF BORIC ACID IN MILK.

From theoretical considerations it is evident that a substance of the character of boric acid would be found also in the milk as well as in the other excretions of the body. Accordingly arrangements were made with a hospital to examine the milk of young mothers shortly after childbirth. The milk was secured by the attendants of the hospital in the usual way. Preliminary examinations were made in all cases to establish the presence of boric acid in the milk. In only one case was it found in the preliminary

examination and this was evidently due to the exhibition thereof at some time prior to the entry of the patient into the hospital.

In the case of the first subject the lacteal excretion was so diminished before the time of the preliminary exhibition of the boric acid as to render the experiment of little value. After five days of preliminary examination, begun on the 26th of June, 1906, 1 gram of boric acid was administered on the 1st of July. The milk which up to this time had given no trace of boric acid, on the first examination thereafter showed a decided trace which, estimated colorimetrically, was equivalent to about one part in 100,000 parts of milk. After the 2d of July the flow of milk became so diminished in quantity that the experiment was discontinued.

In the case of the second subject the preliminary examination of the milk was finished on the 27th of May, 1906. On the 28th of May one gram of boric acid was administered, the same amount on the 29th, two grams on the 30th, and three grams on the 31st, the 1st of June and 2d of June. In this case no trace of boric acid was found in the milk until the 1st of June, when a mere trace was discovered. On the 2d of June, the last day of the administration of the preservative, the quantity excreted in the milk was estimated at one part in 165,000. On the following day, being the first succeeding the cessation of the administration of the boric acid, the amount in the milk had fallen to a mere trace. On the 4th, 5th and 6th of June there was not even a trace. Unexpectedly on the 7th of June, five days after the cessation of the administration of the boric acid a very large quantity was found in the milk, namely, one part in 25,000. On the 8th of June there was one part in 60,000; on the 9th and 10th of June, one part in 90,000 respectively. In this instance it is seen that with the exception of the first day it was not until long after the administration of the boric acid that it appeared in the milk. Immediately after ceasing the administration of the boric acid it disappeared from the milk and did not reappear until the fifth day, when it was present in its maximum quantity, diminishing on successive days until the end of the observation, at which time the amount present in the milk was one part in 90,000.

In the third subject the preliminary examination, which ended on the 27th of June, having extended over four days, exhibited a somewhat remarkable phenomenon, namely, that on the first day

of the preliminary examination, the largest quantity of boric acid which was found at any time in the milk was indicated namely, one part to 3500. On the two succeeding days the amount dropped to a trace, while on the 27th of June, the last day of the preliminary examination, not even a trace was found. One gram of boric acid was exhibited on the 28th and 29th of June, 1⅔ grams on the 30th of June, and 2 grams on July 1st, and July 2d. On the 29th of June the amount of boric acid in the milk was one part in 100,000. On the 30th of June and the 1st of July there was only a trace of boric acid in the milk. On the 2d of July, which was the last day of the administration of the boric acid, there was one part in 100,000. On the 3d of July, which was the last day of the observation, there was one part in 25,000.

These data indicate that appreciable quantities of boric acid administered to the mother are found in the lacteal secretion. The quantity is quite variable and increases or decreases without much relation to the exact date of administration of the preservative. It is evident that the residue of boric acid which is stored in the body may at any time be expected to appear in the milk. Properly, this investigation should have been completed by a study of the animal body itself after the administration of boric acid for a certain period to determine in what organs the part which escapes excretion is principally stored. Theoretically, from the results of the metabolic experiments, a large part of it would be found in the bones, since it was seen that the administration of the boric acid largely increased the excretion of phosphorous. It is our intention in the near future to complete the experiment by feeding animals borax or boric acid for a period of time and then examining their bodies to determine the quantity of borax stored and its distribution.

AIR SUPPLY EXAMINATION.

E. H. RICHARDS.

In considering the practical problems of ventilation, what is needed is the more frequent asking of the question, "Is this air safe to breathe?" as we ask "Is this water safe to drink?"

The readiest means of answering the question should be worked out by this laboratory section.

Some possible methods of arriving at a just conclusion, involve the asking and the answering of the following questions:

I. Is it dusty?

If so, is the dust inert, that is, sand or soot?

Is it composed of active germs?

II. Is it above normal moisture content (humidity)?

III. Is it above the optimum temperature?

IV. Is it odoriferous in any way?

V. Has it above 6 parts in 10,000 of CO_2?

From the results of these inquiries the expert examiner should make his determination, not from any one of them alone. Just as in the case of water supply and food supply there will arise special cases that require special methods. For example, leaky gas pipes fouling the air with carbon monoxide should be detected under IV, odor of gas. Even leaking furnaces give accompanying odors, and sewer gas gives a decided taste on the lips, if not a distinctive odor to the educated nose.

The following are possible methods of answering question I, Is it dusty?

a. Observation by means of a sunbeam crossing the room or by a search light.

b. Placing a glass plate 18 x 24 inches in such a position as to catch the falling dust for two hours.

c. Filtration of from 4 to 10 litres of the air as described below.

d. Examination by means of exposed gelatine plates.

For determining the answer to question II, various instruments are on the market which give humidity readings with no difficulty of manipulation.

To answer III, thermometers cheap and sometimes reliable *can* be procured.

PLATE 4

Apparatus for obtaining dust spots as indicators for air cleanliness.

FUNNEL.

PAPERS.

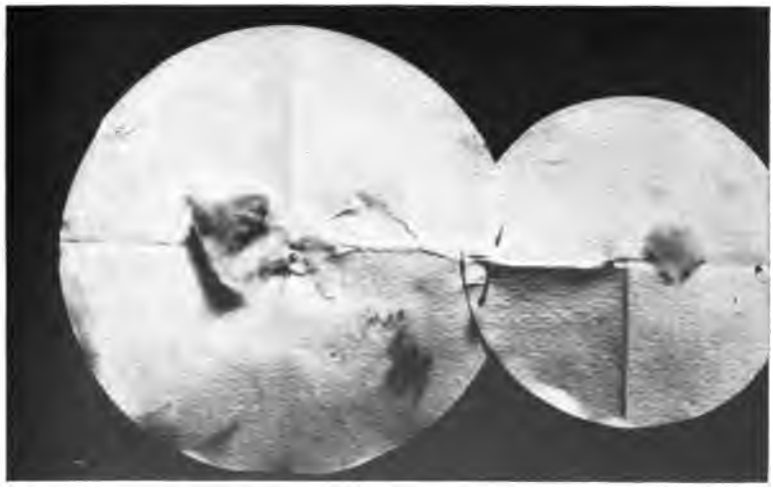


Plate 5

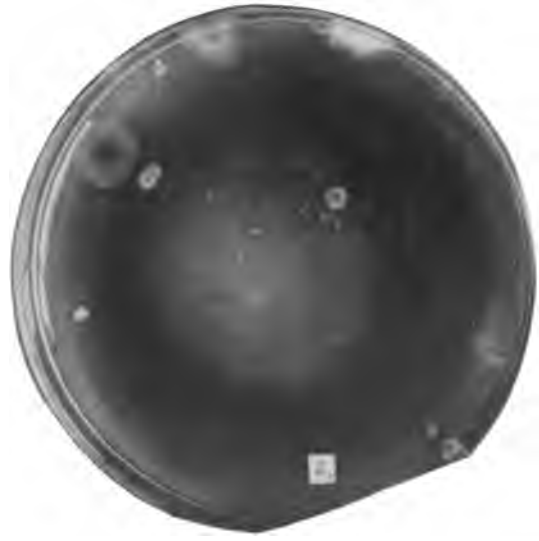
Apparatus for air testing. Cohen and Appleyard principle.

PLATE 6

PETRI DISHES.

No. 2. October 23, 3 p. m., exposed on dining room table for ten minutes, half an hour after carpet sweeper was used on the rugs.

First photograph 48 hours after exposure.



Second photograph 72 hours after.

The test IV requires a certain education of the olfactory nerves and experience in detecting the one odor sought, among several present.

Fifth, the estimation of the CO_2 present should be by one of the approved methods. Modifications of the Cohen and Appleyard method are suggested.

A comparative test for dust in the air may be made by a method similar to that used for milk. A large funnel to serve as director and directing surface is filled with a small, moistened filter paper as for chemical filtration. A small wad of absorbent cotton, which may be moistened with water or glycerine or a sugar solution, is placed in the apex. The air is then passed through by any available means. Suction may be applied from the operator's lungs if he has no other means at his disposal. A length of rubber tubing will even permit him to be in an adjoining room.

The exposure of the Petri dishes may be for such time as seem to answer the particular questions.

It is conceded by all experts that temperature and humidity play a part far greater than that of carbon dioxide in the comfort if not in the health of persons occupying enclosed spaces. Therefore greater attention should be paid to these factors.

The "stuffy" character of air is largely due to odors added to humidity and temperature. Mr. Dissel's tests of railway cars confirms previous work. A greater supply of fresh air is the remedy for all these ills. A keener sense of the foulness of air as it approaches us will tend to health and long life.

A quick and ready method for estimating the carbon dioxide in the air of enclosed spaces is a great desideratum. The properties of the gas and its relations to other substances, have not, hitherto, admitted of a mechanical measure as a thermometer is a measure of the degree of heat.

The Wolpert plan of a bead of liquid flowing down a thread serves for a day or two. But the thread soon becomes encrusted and ceases to conduct the liquid, and the reservoir requires renewing frequently.

The various devices for absorbing the gas by lime water present the most practical methods. The difficulty in manipulation is greatly increased by the improvements in ventilation. Thirty years ago, 20, 30, and even 50 parts of CO_2 in 10,000 were commonly to be

tested for. To-day 6, 8, and 10 are the numbers, so that popular methods are much more difficult to arrive at.

The most scientific and probably the most available method is same modification of the method of Cohen and Appleyard proposed in a paper before the British Association in 1894. It depends upon a scientific fact, (the law of partial pressures), that the greater the acount of CO_2 in a given volume of air, the quicker it will be absorbed by an alkaline solution. Tables may be made for any variations of apparatus used to carry out this principle. For instance, a test of the ventilation of sleeping cars may be made by collecting the air in 500 c. c. bottles and, in the laboratory, noting the time taken to decolorize a lime water solution of a given strength.*

In the examination of places difficult of access or for lecture room experiments, the use of an absorbing tube is very practicable. The volume and *rate* a minute must be strictly noted, together with the strength of solution. Tables prepared for the given conditions enable the operator to estimate closely enough for comparative purposes. The proportions used by the writer are 1-100 of saturated lime water in 20 c. c. volume, at a rate of 100 c. c. a minute.

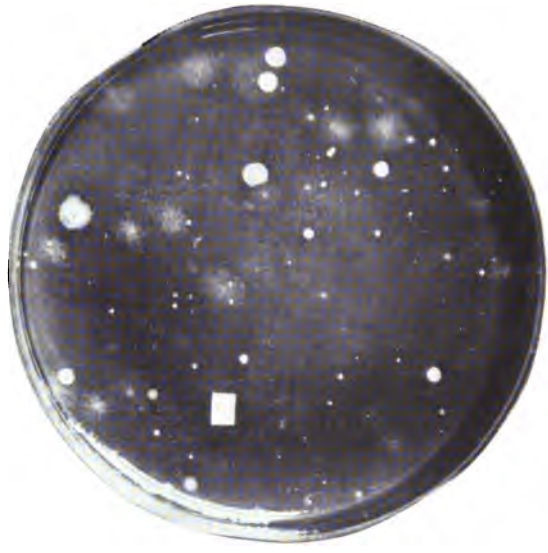
Decolorization is carried only to the decided fading of the phenolphthaline, not to the disappearance of the yellowish pink which is so persistent and which vitiates so much work with this indicator.

* For method and cure see thesis by T. A. Dissel, Course II, M. I. T., 1906.

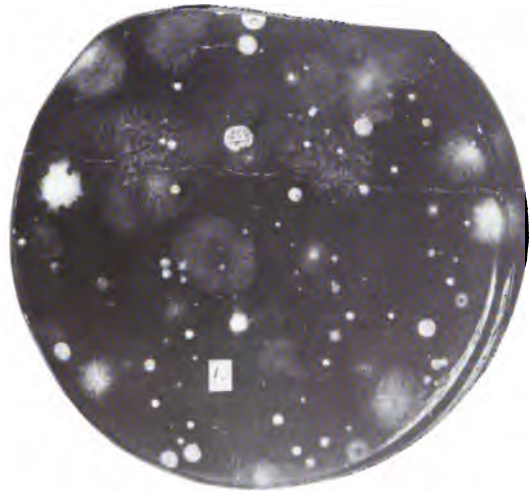
PLATE 7

PETRI DISHES.

No. 1. Reception room. Five minutes exposure during dusting.



Photograph taken 48 hours after exposure.



Photograph taken 72 hours after exposure.

PLATE 8

PETRI DISHES.

No. 3. Library table. Five minutes exposure during dusting and beating up of cushions.

Photograph taken 48 hours after exposure.

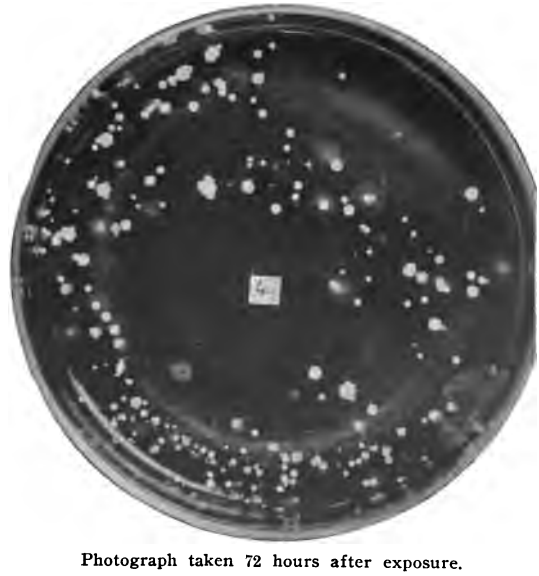
Photograph taken 72 hours after exposure.

PLATE 9

PETRI DISHES.

No. 4. Library table, half an hour after dusting, doors and windows open. 10 minutes exposure.

Photograph taken 48 hours after exposure.



Photograph taken 72 hours after exposure.

TRYPANASOMA LEWISI IN MEXICO.

JOSEPH GIRARD.

Asst. Director National Bacteriological Institute of Mexico.

We desire to call attention to a parasite well known in Europe, the existence of which, we believe, has escaped attention in Mexico. We refer to the trypanasome of the rat T. lewisi.

We believe that this parasite is widely scattered in this city as we have met with it in 62% of the rats examined. This parasite is particularly frequent in young rats.

Trypanasoma Lewisi forms an excellent type for the study of a group of parasites to which belong the grave infections of man and animals (Sleeping sickness, Surra, Nagana, Dourine).

NOTES ON AN EPIZOOTICAL DISEASE IN RABBITS.

DR. OCTAVIANO GONZALEZ FABELA.

In September of last year, a disease appeared in the rabbits that are utilized to conserve the virus of rabies and, in three weeks, 60 animals died.

The animals attacked by this disease only presented lack of appetite,— malaise and diarrhœa; the latter, mucous, greenish and yellowish in character, appearing two or three days before death.

CONCLUSIONS.

1st. In the blood of one of the rabbits naturally ill with the disease in its fatal form, many bodies were found; their morphological characters and staining reactions, mobility and situation, are entirely like those of the piroplasma canis described by Messrs. Noccard and Notas,*

2d. The intravenous inoculation, from this rabbit's blood to other rabbits in series up to the fourth permitted the demonstration in their blood of like bodies, and the appearance of symptoms very similar in character to those of the natural illness, but of diminishing intensity.

3d. The disease, that was in the first inoculated rabbit, acute or subacute, in the second was in benign form, almost chronic in the third, chronic in the fourth, and entirely benign in the fifth.

4th. Diminution in the virulence of the parasite was principally visible by reduction of fever, the scarcity of the hematozoon or a retardation of its evolution and its early disappearance from the blood .

5th. The clinical manifestations were principally lack of appetite, changes in temperature and diarrhœa of variable intensity and duration.

6th. Although this disease has not yet been described, the results of our work, although not so extensive as we would like, warrant the conclusion that a piroplasm, not well known as yet, is the cause of this disease.

* Annale de L'Institute Pasteur, Vol. XVI, p. 267.

DIPHTHERIA BACILLI IN WELL PERSONS IN MEXICO CITY.

DR. OCTAVIANO GONZALEZ FABELA.

For this study, there were made examinations of the products taken from the pharynx of 300 children attending the official schools.

The examinations were carried on during the months from April to August, and practiced on the following conditions:

The culture medium then used was prepared according to the formula of Loeffler:

Simple broth in the proportion of 50 per cent. meat.....	100 c.c.
Sodium chloride	0.50
Peptona	1.60
Glucose	1.00
Sheep's blood serum	300.00

These preparations were made by two proceedings of coloring: that of Neisser, to see the granulations, and that of Gram.

The classification of positive cases was made according to that of Wesbrook.

The age of children to be examined was that from six to ten years and from ten to fourteen, the following results having been obtained:

Number of children examined between 6 and 10 years.....	150
There were found Klebs-Loeffler bacilli in	12
Bacillus exists in 8%	
Number of children examined between 10 and 14.....	150
Diphtheria bacilli were found in.....	11
Bacillus exists in 7.33%	

These should be the figures indicating the proportion of healthy persons in whom the diphtheria bacillus is found, if we take for our calculation every case in which bacilli of any of the three forms appear. The results are very different if we take into consideration only granular forms that appear in clinical cases. We would then make the classification of forms met with in the 23 positive cases as follows:

Type A	3	cases.
Type A′	3	"
Type C′	3	"
Type D′	1	"
Type B	1	"
Type C	6	"
Type D	6	"

If we now make the calculation by classes of bacilli, we shall obtain the following figures in 300 children:

There existed granular forms in 3 cases, or 1%.
There existed barred forms in 7 cases, or 2.33%.
There existed solid forms in 13 cases, or 4.33%.

Then, we may say that, among us, 1% of healthy persons carry diphtheria bacilli.

If we compare this proportion with those obtained in Boston and New York, (2.852 for one city and 6.5 for the latter) we see that, among us, the bacillus is found much less frequently in well persons.

These results are in accord with the fact that diphtheria is much less frequent in Mexico than in Boston and New York.

A STUDY OF HOUSE DISINFECTION AND REINFECTION.

WILLIAM ROYAL STOKES, M. D., and WILBUR P. STUBBS, M. D.,
Baltimore, Md.

The following communication is intended to show the results obtained by the use of formaldehyde gas in room disinfection after communicable diseases, and to demonstrate the effect of this gas upon test cultures. The reinfection of houses following disinfection is also considered. Although disinfection alone is not depended upon to prevent the spread of the communicable diseases, yet we believe that in combination with other means it is useful in limiting their spread. Before stating our results, the methods of prevention in vogue other than disinfection will be mentioned. In order to make a succinct statement the various diseases will be considered seriatim.

TUBERCULOSIS.

A state law of Maryland now requires that every case of tuberculosis shall be reported to the State Board of Health, and when this is done the physician receives a bottle of carbolic acid, a box of sputum cups and a book of directions concerning prophylaxis and cure. In this way the physician becomes the intermediary between the State Board of Health and the patient, and thousands of cases are reached in this way that would otherwise be neglected. The physician is paid a small fee for this service. Compulsory disinfection of tuberculous houses is also required.

DIPHTHERIA.

One of the most valuable adjuncts to disinfection in preventing the spread of diphtheria is the compulsory reporting of cases and placarding of houses. Although not always effectual, yet the warning card puts most people on their guard, and diminishes the exposure to disease, or to healthy persons harboring the bacilli in their throats. Every teacher in each public and parochial school receives a semi-weekly list of all cases of diphtheria reported, and no children from the infected house can attend school until after the disinfection of the house, and the removal of the card from the

door by the health officer. This card is not removed until the throat inspector has visited the house and taken cultures from all of the persons living therein with the exception of the bread-winner. The throat inspector is especially enjoined to take cultures from both nose and throat of the patient and from the throat of the nurse or mother and of each child in the household. If any of these cultures are positive, the throat inspector visits the house every other day and takes cultures, until the throats which contained the diphtheria bacilli are found to be free from these germs.

It may be urged that this custom is not practicable, since it may keep the placard on the house for weeks. Even if such is the case, this entails no very serious consequences. The bread-winners, unless harboring diphtheria bacilli, are allowed free egress and ingress, and the children, deprived of school attendance, no longer serve as sources of general infection to the community.

Occasionally, we find that the diphtheria bacillus remains in the throat for several weeks or more after discovery, or after convalescence. In a series of about 25 such cases we have found that these can be cleared up by the local application of antitoxin. This method was first proposed by Dr. C. Hampson Jones, Assistant Health Commissioner of Baltimore, Md. One thousand units of antitoxin are insufflated through each nostril, and the fluid is allowed to remain in contact with the posterior nares and fauces, as long as possible before swallowing. In most of the cases thus treated the diphtheria bacilli have disappeared from the throat in from one to three days, although a small percentage of cases have still remained positive after this treatment. We have found that this method of throat inspection can be carried out in a city of over half a million inhabitants. Even if a few positive cultures follow the first negative culture, a system based upon this method is better than desuetude.

After all of the cultures are pronounced negative the infected room is disinfected by formaldehyde, and the card is removed from the door. Permits are left at the house, allowing the children to return to school, and the house is relieved of this mild form of quarantine.

The table which follows will show the results of disinfection for 5 years, and while all rooms were not rendered absolutely sterile as far as surface disinfection is concerned, yet the table shows that the majority of rooms were thoroughly disinfected. As far

as the eruptive fevers are concerned, it must simply be assumed that the unknown causes of these diseases were destroyed. The percentage of reinfections from scarlet fever and diphtheria were about the same and we cannot help but feel that disinfection may have helped to limit the spread of the various eruptive fevers. Steam disinfection of mattresses, pillows and other bulky materials is a necessary adjunct to this surface disinfection.

RESULTS OF DISINFECTIONS TESTED BY CONTROL CULTURES.

	1901.	1902.	1903.	1904.	1905.
Positive growth	288	224	336	438	284
Negative (no growth)	832	1018	1733	1606	1508
Empty, broken or not returned	345	136	302	323	185

These figures show that many rooms in Baltimore receive a surface disinfection controlled by a test culture, and that such a method can be greatly improved if the interest of the householders can be further aroused.

THE ERUPTIVE FEVERS.

In our city all cases of smallpox are removed to the quarantine hospital and the house is disinfected by formaldehyde. Cases of scarlet fever are placarded, and such cases as well as measles are reported to the health department, and by this department to the schools.

After three weeks from convalescence if desquamation has ceased the scarlet fever sign is removed and the houses are then disinfected.

METHOD OF DISINFECTION AND CONTROL.

Formaldehyde gas is used for the surface disinfection of rooms, and the method is controlled by means of cultures prepared in the following way: It has been the custom to test each disinfection by placing a dry culture on linen of the staphlococcus pyogenes albus in the room. These linen slips are put into small, sterile tin boxes which are placed in mailing cases consisting of a perforated light board in which the tin box can be placed. The tin box is opened just before the fumigation, and closed and placed in the mailing case as soon as possible after opening up the room. The mailing case is left at the house so that the householder can

return the test culture by mail. The generators usually run 30 minutes for every thousand cubic feet of air-space in the room. The test cultures, when returned to the laboratory by mail, are removed by sterile forceps and at once placed in Dunham's solution. If the medium is not cloudy in 24 hours it is assumed that the culture was destroyed. If cloudy, a microscopic examination will show the coccus if still present.

REINFECTIONS.

The question of reinfections, i. e. second cases of any contagious disease, occurring in the same house, after a proper disinfection, following the primary case, has of late assumed some importance among the various boards of health and has given rise to some discussion.

The first question that suggests itself is what connection there may be between such primary cases and the reinfection. Was the primary case absolutely and entirely responsible for the second case occurring, say, two weeks or a month after the recovery of the primary case? Were all reasonable precautions against a reinfection used? Was the patient isolated? Was disinfection done and if so, was it thorough and efficient? At this point we wish to take exception to the views of those who hold that disinfection is a relic of superstition, and that of no importance in the control of communicable diseases. It is important. We do not mean that a communicable disease (as diphtheria for instance) may be handled in a careless manner during the patient's illness and that disinfection alone will atone for negligence and remove all danger of further contagion. We do say, however, that if precautions are observed during the entire illness, a proper disinfection is not only of some use, but is at this time the most effective agent we have at command to limit the spread of communicable disease.

There appears an article in the Journal of the American Medical Association of Aug. 25, 1906, entitled "The Fetich of Disinfection", in which the author reaches the conclusion: That "Disinfection should not be made so important a part of public health work, and should not be insisted on unless it is practically certain that no member of the household remains infected, and should be refused when it is probable that any member of the household is so affected."

Again he says: "Pathogenic bacteria tend to die rapidly after

discharge from the body." In answer we would like to quote Dr. Osler, who reports a case where virulent diphtheria bacilli were found on a child's toys 5 months after an attack of diphtheria. Experimentally diphtheria bacilli have been kept alive on a piece of silk thread for one hundred and seventy-two days. We do not think the fault lies so much with the disinfection, as with an inadequate quarantine. Unless we disinfect thoroughly we cannot expect good results. In diphtheria, for instance, bits of membrane may be coughed up, deposited on nurse's clothing, and, unless she confines herself strictly to the infected room, this infected material may be accidentally wiped off on furniture in another part of the house. Again, we have seen many convalescent patients, whose throats were charged with virulent diphtheria bacilli, allowed the liberty of the house, distributing diphtheria bacilli from cellar to garret. Should we be surprised at another case developing; and should we lay the blame upon the disinfection? Where the contagion is confined absolutely to one room we may expect good results from proper disinfection. We know, experimentally, that formaldehyde will kill the diphtheria bacillus. If we as health officials refuse, or as family physicians reject disinfection, for the reason that we cannot disinfect in an *ideal* manner, we are making a grievous error. If the ideal goal is unattainabble, there is all the more reason to do our very best.

We have collected statistics from 5546 cases of diphtheria and scarlet fever.

In 2807 cases of diphtheria occurring in Baltimore City, and followed by formaldehyde disinfection, after the throats of all of the household were declared free from diphtheria bacilli, there occurred during the same year, 65 reinfections or 2.35%.

In 2739 cases of scarlet fever followed by formaldehyde disinfections (not less than 21 days after official report of the case to the Baltimore City Health Department) there were 69 reinfections or 2.55%.

In 1769 of the above diphtheria disinfections, there were 40 reinfections where we have obtained the result of the control cultures. Three of these were positive, showing the disinfection for some reason (construction of room, etc.) was technically defective. Thirty-one were negative, showing a thorough disinfection, and six were either missing, sent back empty or were broken in transit.

In 1721 of the above scarlet fever disinfections, there were 47 reinfections, of which we have obtained the result of the control cultures. Seven were positive, 32 were negative, and 8 were missing or empty.

We will next show in a series of cases, the lapse of time between the fumigation of the primary case and the report of the second case, i. e., date of reinfection. In 1850 of the above diphtheria cases from which we have been able to gather this information there occurred 47 reinfections (2.54%).

The following table shows the lapse of time between the disinfection of the primary case and the reinfection.

8	reinfections	occurred	within	1	week.
13	"	"	"	2	weeks.
6	"	"	"	3	"
5	"	"	"	1	month.
3	"	"	"	2	months.
5	"	"	"	3	"
4	"	"	"	4	"
1	reinfection	"	"	5	"
1	"	"	"	6	"
1					
47	total number of reinfections in 1850 diphtheria disinfections.				

In 1571 cases of scarlet fever there were 35 reinfections or 2.23%.

14	reinfections	occurred	within	1	week.
11	"	"	"	2	weeks.
1	reinfection	"	"	3	"
4	reinfections	"	"	1	month.
3	"	"	"	2	months.
1	reinfection	"	"	3	"
1	"	"	"	4	"
35	total number of reinfections in 1,571 scarlet fever disinfections.				

Just what relation exists between the primary and secondary cases in these tables is a question that could be discussed at some length. We have not the time to enter into it here. At best it would be only a matter of surmise. We will say, however, that the difference in time between the diphtheria and scarlet fever reinfections is about what we would expect when we take into consideration the clinical characteristics of the two diseases. The period of incubation is as a rule longer in scarlet fever than in diphtheria, and, what is more important, the period of infection is

more prolonged in scarlet fever than in diphtheria. In a scarlet fever case, complicated with an otitis, rhinitis, suppurating glands, etc., who can say just how long the infective period may last? In the 25 reinfections occurring within two weeks after disinfection of the primary case, we would have just ground for supposing that the patient was exposed to the same source of contagion as was the primary case, or that the secondary infection was inflamed by the primary case, prior to disinfection.

In the article quoted above (Fetich of Disinfection), the author states that he has been so impressed with the futility of disinfection that as chief health officer of his city he has refused disinfection following diphtheria unless certain conditions are met by the patient, which he admits very rarely happens. He cites 258 cases of diphtheria which were not followed by disinfection. In these cases there was 1.55% reinfection in *two months*. This average for the whole year would at the same ratio of reinfection mean 9.30% reinfections in these 258 cases for the year. Against these figures we have 2807 cases, each followed by a thorough disinfection with 2.35% reinfections for the entire year. We recognize that this in all probability is not a fair way to average the reinfections, as certainly a larger proportion of reinfections would occur in the first two months than in any subsequent two months, and that the proportion of reinfections per month would probably decrease in reverse ratio to the length of time after the primary disinfection. All this being true 2.35% reinfections for the period of 12 months would seem eminently better results than 1.55% reinfections in 60 days.

THROAT INSPECTION.

We believe in the efficacy of throat inspection in all cases of diphtheria prior to disinfection. Frequently we have found the inmates of an infected (diphtheria) house harboring virulent diphtheria bacilli in their throats, without showing any clinical symptoms whatsoever. The laboratory is absolutely the only place where such foci of infection can be recognized. These "carrier-cases" may at any moment break out into malignant cases of diphtheria, or worse yet, may contaminate some of those with whom they come into daily contact and who for some reason enjoy less natural resistance to this particular disease. The following table shows the number of throat cultures taken in Baltimore City during the past four years. These cultures were taken after all clinical symptoms had

subsided, and after the attending physician declared his belief that all danger of contagion had passed, and that the premises were ready for fumigation.

Year.	1902.	1903.	1904.	1905.
Number of cultures taken	1915	4649	4703	3601
" " negative	1908	4278	4310	3437
" " positive	97	371	93	164

When we take into consideration that among the positive cultures enumerated above, not one was taken for diagnostic purposes, but were all taken from throats supposed to be free from infection, it is absurd to expect any other than injurious results if these several hundred nests of infection were allowed to roam at will among the inhabitants of a thickly populated city.

For the sake of illustrating the importance of throat inspection, and incidentally what one infected throat can probably do, one history will suffice. There is in Baltimore a society called the Fresh Air Society, which sends children to the country for a few weeks in the summer. In September, 1903, there was located some few miles from Baltimore City a camp of fifty-one of these children. One child complained of a sore throat, and several later developed clinical diphtheria. One of the writers was sent to the camp, and after making throat cultures of the fifty-one children found seven of them containing living diphtheria bacilli. This camp was situated a mile or more from any town or village and it was somewhat difficult to trace the source of the contagion. We discovered that the man who supplied the camp with milk had a case of diphtheria in his own family a few weeks earlier. We took a culture from his throat, and found almost a pure culture of the diphtheria bacilli. That he was responsible for the slight epidemic at the camp would be impossible to prove, but circumstantial evidence seemed to indicate that such was the case.

Prior to July, 1898, the Baltimore City Health Department did not practice routine throat inspection. The following table shows the decrease in the number of diphtheria cases reported since 1898.

Year.	1898	1899	1900	1901	1902	1903	1904	1905
Number of diphtheria cases..	2019	1783	1858	1149	941	1436	1241	941

When we take into consideration the increase in population from 541,000 in 1898 to over 600,000 in 1906 it makes the above figures more striking.

It may be urged that the decrease in the number of cases of diphtheria was due to a gradual weeding out of the most susceptible, and on examining the cases reported previous to 1898 such might seem to be the case, — since the number of cases reported yearly is frequently smaller than those reported during the years cited in our statistics. The general stirring up of the profession by placarding houses, more thorough disinfection, and a general campaign against diphtheria, increased greatly the number of cases reported, and since 1898 we believe that the vast majority of cases have been reported.

The continued restraint of diphtheria during the years succeeding 1898 suggests that this decrease was partly caused by the methods described above, but it will require further study to prove this fact.

In conclusion we hope to have shown: (1) The necessity of a thorough disinfection, (2) That disinfection properly carried out and at the proper time is one of our most effective agents in checking the spread of contagious diseases, (3) The number of reinfections following a thorough disinfection is comparatively small, (4) The importance of throat inspection prior to disinfection in diphtheria.

OBITUARY.

CHARLES A. SNODGRAS, M. D.,

OF ST. LOUIS.

Dr. Charles A. Snodgras was born January 2nd 1864, in Jackson County, Missouri. His father, William A. Snodgras, and mother, Mary Snodgras are living at an advanced age at Independence, Mo.

Dr. Snodgras' earlier education was obtained at the Missouri State University, and on leaving the university he accepted a position as teacher in a small school in Saline County, Missouri. While teaching there he was selected to become principal of the high school at Marshall, Missouri. During the summer seasons Dr. Snodgras attended the sessions of the Chicago University in order to perfect himseif in the branch of learning which afterwards became his life work. As a result of this training he was made head of the department of science in the high school at Fort Smith, Arkansas, and remained there for two years; during which time in vacation periods he returned to the University of Chicago for further study.

In 1898 Dr. Snodgras came to St. Louis and was attached to the department of anatomy of Washington University. His indomitable courage and close application to the study of medicine while teaching there enabled him to take the degree of Doctor of Medicine at that University in the year 1904.

As a bacteriologist Dr. Snodgras did some very valuable work in the recent suit of the city of St. Louis regarding the Chicago drainage canal, his investigation being complete and convincing to men of science.

In 1903 Dr. Snodgras was appointed city bacteriologist by the mayor of St. Louis, in which position he worked unremittingly until his promotion to the position, first, of acting health commissioner and finally — on the removal of the former incumbent — health commissioner of the city of St. Louis.

One of the most important of his official acts as Health Commissioner was, with the aid of architects, the designing and erection of the new municipal laboratory of pathology and bacteriology, which stands to-day one of the most complete of its kind from the

standpoint of both general appointments and scientific equipment that exists on the American continent.

While principal of the high school at Marshall, Missouri, Dr. Snodgras married Miss Anna Gamble. Two children, Alvin, aged five years, and Dorothy aged three years, together with his wife, remain to mourn his untimely death, which occurred as a result of double pneumonia on Friday, April 6th, 1906, at the City Hospital of St. Louis, notwithstanding the unremitting attention of his professional colleagues.

Dr. Snodgras was a man whose mind had never been contaminated by anything small or selfish. He was just and rigorously honest. Upon his promotion to the office of health commissioner his entire energy was devoted to a complete study of the various departments under him, in order that, before attempting any improvement, he might be thoroughly familiar with every detail. To accomplish this purpose he gave each and every department careful and consistent study. As a result of his energy many important innovations have been begun. It was through his efforts that the establishment of the present tuberculosis clinic has been effected at which those unfortunates afflicted with this grave disease are not only treated but educated in the methods which have for their object not only the benefit of the individual but the protection of those who come in contact with him. In having diphtheria antitoxin supplied to all worthy poor and administered by the physicians connected with the dispensary department is another result of his sagacious work. The fight begun by the St. Louis Medical Society against irregular and irresponsible practitioners of medicine found in him a worthy ally and one without whose aid the society would have encountered unsurmountable difficulties.

In the space of this brief obituary it is indeed a difficult task to enumerate the many accomplishments of the man as well as the various plans that he had in view for the advancement of public health. He possessed the confidence of each and every one of his subordinates. His simplicity of manner, his open-heartedness, made them, one and all, feel that in him they had a superior officer with whom they could consult and confide and that ideas or suggestions made to him found always ready and careful attention. In his death the medical profession loses a valuable member, the city of St. Louis an honest, upright and conscientious servant,

and his family a devoted and loving husband and father. Though his public life was indeed short, yet it stands out clearly and boldly. It serves to set a standard that those who follow him should strive to emulate. If naught but this were left by him as a heritage his life has served a noble purpose. H. WHEELER BOND.

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